GAO

United States General Accounting Office

Report to the Honorable
Robert L. Livingston,
House of Representatives

December 1990

ARMY BIOMEDICAL RESEARCH

Concerns About Performance of Brain-Wound Research

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GAO United States
General Accounting Office
Washington, D.C. 20548

Human Resources Division

B-233716

December 12, 1990

The Honorable Robert L. Livingston
House of Representatives

Dear Mr. Livingston:

In response to your request, this report presents the results of our review of brain-wound research by the Louisiana State University (LSU) School of Medicine in New Orleans under contracts with the U.S. Army Medical Research and Development Command.

Experts we consulted have concerns about the performance of the research that raise questions about the validity of some of the results. In addition, we have concerns about the Army's management of its contracts with LSU for this research.

This report contains recommendations to the Secretary of Defense; they are designed to ensure that his decision on the remaining contract's future is based on a determination of whether or not the project will provide additional useful information and, if so, that the concerns we identified have been resolved.

As agreed with your office, unless you publicly announce its contents earlier, we plan no further distribution of this report until 7 days after its issue date. At that time, we will send copies to certain House and Senate Committees and to other interested members of the Congress. We are also sending copies to the Department of Defense; the Department of the Army; Dr. Michael E. Carey, Louisiana State University School of Medicine in New Orleans; and other interested parties.

This report was prepared under the direction of Linda G. Morra, Director, Intergovernmental and Management Issues. Please call her on (202) 275-1655 if you have any further questions. Other major contributors to this report are listed in appendix XIII.

Sincerely yours,

Lawrence H. Thompson

Lawrence H. Thompson
Assistant Comptroller General

the contract requirements; the panel also provided individual comments to a series of questions GAO developed. Following the medical panel meeting, GAO consulted several veterinary anesthesiologists.

The overall goal of the LSU research is to better understand the pathophysiology (alterations in the physiological functions produced by a disease or pathological process) of fragment wounds to the brain and develop a drug protocol that can be used in conjunction with surgery to treat such wounds. GAO's panel chairman summarized it thus:

> The problem of missile injury both on the battlefield and in civilian circumstances is important. Understanding the pathophysiology of missile injury is the only way that progress can be achieved in treatment.

Results in Brief

GAO's medical panel was generally positive about the research. The panel believes that research in this area is needed, no one else is working in this particular area, and the model is unique for studying brain wounds. The panel concluded that the project had merit and funding should continue. Most panelists, however, expressed concerns about the research in two areas—the management of general anesthesia and post-operative care—that they thought could affect some research results. GAO therefore concentrated on these areas in its review. For assistance, GAO consulted five veterinary anesthesiologists.

GAO asked the veterinary anesthesiologists to review the information provided to the panelists, as well as additional information developed by GAO, and focus on the areas of concern. The five veterinary anesthesiologists had several concerns that raised doubts about the validity of some of the research results. Specifically, all of the anesthesiologists questioned the management of the general anesthesia; the management of postoperative care; and, in reported results, the exclusion of data on large numbers of animals used in the research. Several of the anesthesiologists also questioned the reliability of blood gas measurements and the number of animals used that did not result in usable data. (See ch. 2.)

GAO also determined that the research does not violate the public law limiting the use of cats and dogs in DOD projects. The law pertains to the medical training of DOD personnel, not research efforts. (See ch. 3.)

Finally, GAO determined that the Army's management of the research contracts has been inadequate. The Army did not follow its standard critical to the outcome of the LSU study—such as, cerebral (brain) blood flow and cerebral metabolism—are influenced by general anesthesia. The changes in blood flow and metabolism are directly related to the anesthetic dose. Therefore, unless the dose is precisely controlled, the veterinary anesthesiologists GAO consulted said, it is impossible to determine whether the pathophysiological changes are due solely to the injury or to a combination of the injury and the anesthesia. These anesthesiologists were in agreement that with the particular anesthetic and its method of administration in the LSU research—pentobarbital injected first into the cats' abdominal cavities, followed by intravenous injections as needed—the depth of anesthesia was difficult to control.

GAO found that anesthesia doses and the times they were administered were recorded for only 20 to 25 percent of the animals used in the research. On the basis of a review of the anesthesia records GAO obtained from LSU researchers, the veterinary anesthesiologists doubted there was comparability in the depth of anesthesia among cats used in the experiments. (See ch. 2.)

Questions About Postoperative Care

Postoperative care for animals allowed to awaken from anesthesia is important in order to interpret physiological and behavioral changes that may be caused by experimental procedures, such as injury, or by anesthesia or pain. Further, standardized postoperative care procedures are needed to ensure that research data for all animals used in the research are comparable. However, the research team did not consider postoperative care factors important to the research. The veterinary anesthesiologists GAO consulted identified several factors that suggest deficiencies in postoperative care, such as the lack of detailed records to confirm that uniform care was provided to all animals. The veterinary anesthesiologists pointed out that careful management of the postoperative period (that is, monitoring such factors as body temperature, fluid balance, and reflexes) is important to distinguish between the recovery of treated and untreated animals to identify effective drug treatments. GAO was not able to obtain sufficiently detailed records to answer the anesthesiologists' questions about postoperative care. (See ch. 2.)

Questions About Other Areas of Research Performance

The veterinary anesthesiologists raised additional questions on information related to other areas of research performance. Of particular concern were

DOD and LSU disagreed with GAO's observations on scientific issues related to

- control of general anesthesia and its potential effect on research results;
- the effect and adequacy of postoperative care; and
- other aspects of research performance including questions about (1) the possible recording of measurement errors in blood gas values, (2) the ability of the trauma model to produce predictable graded responses, (3) failure rates during the performance of the project, and (4) concerns about data-reporting methods.

LSU also disagreed, in part, with the process GAO used to conduct its review.

GAO believes that both the process used to conduct its review and the concerns raised are valid. Differences of opinion exist on the scientific issues discussed in this report; GAO's recommendations were intended to focus DOD's attention on these issues as it decides whether to continue the LSU project.

GAO's more detailed response to the DOD and LSU comments appears in chapter 4. The full text of the DOD and LSU comments are presented in appendixes XI and XII respectively.

Glossary

Tables

Figures

Abbreviations

AAALAC	American Association for Accreditation of Laboratory Animal Care
BBB	blood-brain barrier
COR	contracting officer's representative
CRISP	Computer Retrieval of Information on Scientific Projects
CSF	cerebrospinal fluid
DOD	Department of Defense
DVM	Doctor of Veterinary Medicine
E	energy ($E=1/2mv^2$)
ICP	intracranial pressure
IM	intramuscular
IP	intraperitoneal
IV	intravenous
LSU	Louisiana State University
MABP	mean arterial blood pressure
MEDLINE	Medical Literature Analysis and Retrieval On-Line
mm/Hg	millimeters of mercury
VMD	Veterinary Medical Doctor

Introduction

In the summer of 1988, amid growing concerns about the proper use of animals in biomedical research, a project funded by the Department of the Army achieved national attention. The project entailed the injuring of cats to study shell and other fragment wounds to the brain. Details of this research, conducted at the Louisiana State University (LSU) School of Medicine in New Orleans, were made public in September 1988 as the result of a Freedom of Information Act request; then the details rapidly surfaced in the media.

The Army defends the project as necessary to learn how to better treat combat-incurred brain wounds so that injured soldiers can be returned to duty and thereby conserve military fighting strength. Critics of the research, including the Louisiana Society for the Prevention of Cruelty to Animals and New Orleans-based animal welfare groups, argue that the project violates federal law limiting use of dogs and cats in DOD projects; in addition, the project is unlikely to expand the body of knowledge established by other research on the treatment of brain wounds.

Given these differing views of the value of the LSU research, Representative Robert L. Livingston asked GAO, in an October 28, 1988, letter, to review the research project to determine whether it can be expected to provide useful results. He also asked GAO to review the Army's process for approving and monitoring its contracts with LSU and determine whether the contracts violate the public law limiting the use of cats and dogs in DOD projects.

In the past year, the LSU research project has generated widespread congressional concerns, generally similar to those of Representative Livingston. As a result, the Defense Appropriations Act of 1991 (P.L. 101-511, Nov. 5, 1990), prohibits the Army from disbursing any of its fiscal year 1991 or prior years' appropriations to fund the LSU research, except for previously incurred costs, pending completion of GAO's review.

Background

The U.S. Army Medical Research and Development Command conducts a medical research and development program designed to support the soldier in the field and meet other Army health needs. Research focuses on combat casualty care, military disease hazards, combat weapon systems hazards, and chemical weapons defense. The LSU project emphasizes the significance of brain-wound research to care for combat casualties. Project proposals indicate that although 40 percent of all combat deaths are from brain wounds, many soldiers survive such wounds. The proposals add, however, that almost one-third of the Army servicemen who received brain wounds from missiles in World War II and the Vietnam War were able to continue some form of duty.

The purpose of the LSU research is to enhance the understanding of brain wounds, thus enabling combat physicians to effect better drug treatment. The research proposals state that it is unlikely that further development of medical evacuation, facilities, equipment, and supplies for the treatment of injured soldiers—which were optimal during the Vietnam War—will save lives. The proposals also indicate little prospect for reducing the death rate through improved neurosurgical techniques.[1] The proposal (see proposal I, app. I), which resulted in the first of two contracts with LSU, proposes research that

> . . . will provide the first steps in providing a comprehensive delineation of the pathophysiology of brain wounding caused by conventional weapons and optimal treatment. Hopefully, knowledge gained will result in a significant reduction of wartime neurosurgical mortality from 10 to perhaps 5 percent or less. . . . This project is designed to provide information immediately transferrable to the clinical setting.

Research Objectives

The LSU research focuses on wounds caused by low-energy missile shell and other fragments. On the basis of an unsolicited proposal, LSU was awarded a sole-source contract, on June 22, 1983, for research, entitled "The Effects of an Experimental Missile Wound to the Brain on Brain Electrolytes, Regional Cerebral Blood Flow and Blood Brain Barrier Permeability; The Treatment of the Resultant Disorders" (Contract DAMD17-83-C-3145). (See Glossary for definitions of terms in this title.) Before the contract was awarded, the Army had the opportunity to comment on the research proposal. In addition, the proposal went through a formal peer review process.[2]

The research objectives, as stated in the proposal, were to

[1]Neurosurgical mortality of combat-incurred brain wounds for U.S. forces was 14 percent in World War II, 9.6 percent in the Korean War, and 10 to 12 percent in the Vietnam War. LSU's principal investigator views these data as indicating that no reduction in brain-wound mortality for U.S. forces has taken place over the past 35 years (see proposal II, app. II).

[2]In the peer review process, proposals are competitively evaluated through a discussion conducted by a review committee composed of scientists knowledgeable about the proposal subject. The committee evaluates each proposal to determine its scientific acceptability in areas such as research objective, scientific feasibility, investigator competence, and animal use.

- document the acute changes in (1) brain water and electrolytes, (2) regional cerebral blood flow and cardiac output, and (3) blood-brain barrier [BBB (see Glossary)] permeability consequent to a nonfatal missile wound in cats and
- test three drugs given 1 hour after wounding to determine whether they minimized or prevented physiological dysfunction of the variables listed above.

Following contract modification (BBB work was deleted) the period of performance for the first contract was from July 1, 1983, to December 31, 1985, at a cost of $342,450.

In response to LSU's January 30, 1985, proposal, a follow-on contract was awarded April 15, 1986, entitled "Experimental Study on a Brain Missile Wound; Ascertaining Pathophysiology and Evaluating Treatments to Lower Mortality and Morbidity" (Contract DAMD17-86-C-6098). Peer-reviewed in June 1985, the proposal was incorporated in its entirety into the contract. Research conducted under the second contract is designed to develop "sound physiologic and pharmacologic methods to ameliorate brain damage" by concurrently

- studying the neurological status of the animals before and after gunshot wounds to the brain to assess which drug treatment results in decreased mortality and morbidity and
- comparing the pathophysiology of wounded untreated cats (control cats) to wounded cats subjected to treatments (study cats) shown to be effective in reducing mortality and morbidity.

The period of performance specified in the contract—April 14, 1986, to September 29, 1991 (revised from the original proposal)—has remained unchanged to date; however, through contract modifications, the cost has increased from $1,681,773 to $1,767,894. As of August 27, 1990, a total of $1,351,669 had been paid to LSU under this contract.

With the exception of budget data deleted by the Department of the Army and the personal information deleted by GAO, appendix I provides the complete proposal for the first contract and appendix II provides the complete proposal for the second contract.

LSU is accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). AAALAC is an organization that accredits institutions engaged in animal research. Institutions voluntarily seek accreditation, which, if obtained, must be periodically renewed.

Trauma Model

A trauma model in biomedical research consists of the animal used, the method of preparing the animal for injury, and the method of inflicting a physical injury so as to study the effects of the trauma on the animal or assess the efficacy of drug treatments or both. An essential characteristic of a valid trauma model is its repeatability; that is, the model can be used to produce an injury predictably and consistently. The trauma model for the LSU research is an anesthetized cat, shot in the head with a specially designed "gun."

The proposals indicate that cats were selected because (1) their brains have a ratio of gray to white matter comparable with that of human brains and are small and would not, therefore, require large amounts of expensive radioisotope (radioactive isotope used as a tracer to follow the course of blood flow) doses and (2) they are readily available and relatively inexpensive.

The method of preparing the animals for injury is generally the same in proposals I and II, except for the anesthetic regimen and trajectory of the missile into the brain. The animals are placed under general anesthesia;[3] monitors are used to measure various physiological parameters, such as blood pressure, hematocrit (the percentage of the whole blood cells in relation to the plasma content), and arterial blood gases (oxygen and carbon dioxide concentrations found in blood). In proposal I, the anesthetic protocol includes halothane (an anesthetic administered by inhalation), methohexital sodium (an ultrashort-acting barbiturate sold under the trade name of Brevital), and nitrous oxide (a colorless gas used to produce anesthesia); in proposal II, the anesthetic protocol includes methohexital sodium and nitrous oxide. In proposal I, the missile (a steel sphere 2 millimeters in diameter and weighing 31.7 milligrams) enters the left temple and follows a path from left to right across the brain behind the eyes. In proposal II, the anterior (front) wall of the right frontal sinus is surgically removed to facilitate penetration of the skull by the missile, which inflicts the injury front to back in the right cerebral hemisphere. In both proposals, the animal's head is positioned and immobilized in a device called a stereotaxic frame.

[3]General anesthesia is defined as a state of altered body function resulting in insensibility to pain and a loss of consciousness. It is accomplished either by the injection of a combination of drugs or a single drug or by the inhalation of an agent combined with oxygen. Drugs injected alone or in various combinations to produce a state of general anesthesia include narcotics, tranquilizers, dissociative agents, and barbiturates, such as the pentobarbital used in this study. Injections may be intraperitoneal—into the abdominal cavity—intravenous—into a vein—or into a muscle mass. Inhalation agents are delivered to the animals' lungs in a carrier gas, such as oxygen or an oxygen-nitrous oxide mixture. The animal inhales this mixture through a mask placed over the face or through a tube (known as an endotracheal tube) inserted into the trachea (windpipe).

The protocols indicate that study animals are then shot at one of three energy levels—0.9 joules, 1.4 joules, and 2.4 joules.[4] Depending on the nature of the specific experiment performed, in proposal I, physiological parameters are monitored for times ranging from 0 to 360 minutes (6 hours) before the animal is euthanized (put to death easily and painlessly). In proposal II, physiological parameters are also monitored for times ranging from 0 to 360 minutes, and the behavior of cats receiving drug treatments is assessed daily for 21 days after wounding. Animals allowed to survive for more than 6 hours are returned to LSU's animal care facility. At the end of the experimental procedure, the animals are reanesthetized before being euthanized.

The gun used to inflict the fragment wound consists of a precision-made steel tube seated inside a 69-centimeter (100th of a meter) hollow steel bar. The inside diameter of the steel tube is just large enough to accommodate the missile that inflicts the injury. The barrel is coupled to a valve that controls the release of pressurized helium propellant. Velocity of the missile is a function of helium pressure released by the valve. The velocity is determined by the time required for the missile to pass between two electronic break screens set exactly 50 centimeters apart.

Research Results

As of June 1989, the LSU research team had worked on 33 experiments, each of which is described in appendix III. In a January 3, 1989, letter to the Army's contracting officer's representative (COR), the principal investigator stated that the LSU research effort has resulted in the following unique accomplishments:

- establishment of a trauma model in the anesthetized cat where a realistic brain wound is made through the intact skull;
- reemphasis of existing knowledge that the missile's crushing effect on the brain may not be responsible for its lethal effect; rather, it is the indirect effects of the missile acting on the brainstem, many centimeters away, that is lethal by causing respiratory arrest;

[4]Joules is a unit of energy (that is, the capacity to do work). The energy in joules of the sphere is calculated by $E = 1/2\ mv^2$, where "E" represents energy, "m" represents the mass (in kilograms) of the sphere, and "v" represents velocity (in meters per second) of the sphere. Using a 31.7 milligram steel sphere, consistent bone penetration of the posterior wall of the right frontal sinus required an energy level of 0.9 joules. At 1.4 joules, the wound was fatal due to immediate respiratory arrest in about 40 percent of all the animals; at 2.5 joules, to 66 percent. Because the interest was in pathophysiology of nonfatal brain wounds, three discreet energy levels—0.9 joules, 1.4 joules, and 2.4 joules—were selected to produce graded responses for acute, subacute, and long-term physiologic changes.

- demonstration that something as simple as cardiopulmonary resuscitation may dramatically lower the mortality from a brain wound;
- determination that respiratory failure [apnea] accounts for the immediate mortality from missile wounding to the brain even though the missile comes nowhere near the respiratory centers in the brainstem;
- determination of the normal recovery time and recovery pattern for motor defects [paralysis] following brain wounding in the untreated cat;
- demonstration that missile wounding disrupts the so-called blood-brain barrier [BBB], which must be intact for normal brain function, not only around the wound track but at a distance from the missile track;
- computation of the time course and magnitude of post-wounding brain edema [swelling] for the missile injury to the brain;
- demonstration of the enormous increase in prostaglandins [powerful hormone-like chemicals that affect the nervous system] in the cerebrospinal fluid within minutes after wounding;
- study of regional blood flow throughout the brain ascertaining that (1) brain wounding is not associated with a lessening of blood flow [ischemia] either about the wound track or anywhere else in the brain, (2) a missile wound to the brain followed by simultaneous, major blood loss in other parts of the body may lead to severe loss of brain blood flow that is not restored by infusion, (3) the missile-wounded brain loses its ability to control its own blood flow through chemical blood flow autoregulation, and (4) increased levels of oxygen in the blood decrease blood flow to the brain after missile wounding; and
- determination that a missile wound to the brain affects not only the brain, but causes systemic effects as well, such as increasing plasma catecholamines [any one of a group of natural substances released by the body as a result of stress or injury].

Objectives, Scope, and Methodology

As agreed with Representative Livingston, our objectives were to carry out a detailed assessment of the LSU brain wound research to (1) determine whether it will provide useful results and (2) review the Army's management of its contracts with LSU. We also agreed to determine whether the research violates section 8056 of the DOD Appropriations Act of 1988 (P.L. 100-202), which is the federal law that pertains to DOD's use of dogs and cats in DOD projects.

To accomplish these objectives, we

- reviewed the Army's contract files—including the research proposals, quarterly and annual progress reports submitted by LSU on the first and second contracts, the final report submitted on the first contract, draft articles prepared by the principal investigator for submission to peer-review journals, correspondence between LSU and the Army—and documentation pertaining to requests made under the Freedom of Information Act for information on the research;
- reviewed section 8056 of P.L. 100-202 and its legislative history;

- interviewed Army officials responsible for approving and managing this research at headquarters—Army Medical Research and Development Command, Fort Detrick, Maryland—and Letterman Army Institute of Research, San Francisco, California;
- visited the LSU School of Medicine in New Orleans to discuss the project with LSU officials and the research team, inspect the laboratory and the animal care facility, and review laboratory notebooks on the experiments performed;
- met with representatives of the Physicians Committee for Responsible Medicine—an organization based in Washington, D.C., that advocates using alternatives to animals in research—and reviewed the critiques of the research project they provided;
- convened a medical panel to review and evaluate the scientific aspects of the research and identify areas, if any, warranting further investigation;
- consulted veterinary anesthesiologists to review reported information in the research areas in which the panel had concerns;
- conducted a literature search of the MEDLINE (Medical Literature Analysis and Retrieval On-Line), Defense Technical Information Center, and CRISP (Computer Retrieval of Information on Scientific Projects) data bases to identify ongoing or completed research involving brain wounds, cats, or anesthetics and various physiological parameters relevant to the LSU project.

The members of our medical panel were selected to provide expertise in such relevant areas as neurosurgery, neurology, anesthesiology, trauma, anatomy, and veterinary medicine. Initially, we selected members on the basis of recommendations from GAO's chief medical advisor, the director of the National Institute of Neurological and Communicative Disorders and Stroke, the American Medical Association, and the Physicians Committee for Responsible Medicine. If the recommended member could not assist us because of a scheduling conflict, we asked that he or she recommend a substitute with similar expertise. The members of our medical panel are listed in appendix IV.

Before the meeting, we sent the panel members information on the research project, including the two research proposals, the annual report and final reports on the first contract, the annual reports on the second contract (covering research completed through April 1988), and the two quarterly reports on research completed from April through October 1988 (the last progress reports LSU submitted to the Army). Also before the meeting, we asked the panel chairman to review and comment on a draft of questions we prepared to stimulate the discussion and address the issues raised by the Physicians Committee for Responsible Medicine. He accepted the questions as submitted and made preliminary assignments of specific questions to each panel member on the basis of his or her area of expertise.

At the panel meeting, held at GAO on June 19, 1989, we provided information on the equipment used at the LSU research facility (much of which was purchased with Army and DOD funds); the curriculum vitae of four members of the research team, current as of June 1989; and data from the laboratory notebooks on research protocols for 33 experiments, including the numbers of animals used and the types and amounts of anesthetics given.

In the meeting, the panel discussed the project's goals, methodology, and value; the trauma model; animal care; anesthetic controls; and investigator qualifications and equipment. The discussions in each of these areas focused on both the research as proposed and the research as performed. Further, in each area, the panel discussed specific questions we had prepared. At the end of the discussion for each area, and before moving into the next area, the chairman asked the panelists to write their responses to the questions in workbooks we provided (see app. V).

Immediately after the June 1989 meeting, we reviewed each panelist's written comments and identified general areas of concern: control of general anesthesia and postoperative care. Because six of the eight panel members expressed concerns about some aspect of the anesthesia proposed or used in the research and its effects on the results, we consulted veterinary anesthesiologists about anesthesia and its effects on cats. To identify these panelists, we obtained recommendations from both the Assistant Deputy Administrator for Animal Care for Regulatory Enforcement, U.S. Department of Agriculture, and a coeditor of Veterinary Pharmacology and Therapeutics[5]; we also reviewed the references cited in the 5th and 6th editions of this reference work. The veterinary anesthesiologists that we consulted, who have themselves done animal research, are listed in appendix VII.

On September 13, 1989, we visited LSU and briefed the principal investigator on our preliminary findings. During that meeting, he provided additional information concerning the experimental protocols, anesthetics used, and postoperative care. Further, following the meeting, at

[5]Nicholas H. Booth and Leslie E. McDonald, eds., Veterinary Pharmacology and Therapeutics, 6th ed. (Ames, Iowa: Iowa State University Press, 1988).

his request, we sent each member of the medical panel (1) the abstracts of papers that research team members had presented on the research and (2) a draft of an article on the research subsequently published by the Journal of Neurosurgery.[6]

After the meeting at GAO in June 1989, the panel chairman reviewed and drafted a summary of the individual comments and the panel's discussion; the chairman circulated this draft summary to each panelist for review. GAO received the panel's final report on October 23, 1989 (see app. VI).

We met again, at GAO headquarters in Washington, with the principal investigator and other LSU officials on November 9, 1989. At that meeting, LSU provided additional information on the anesthetic aspects of the research, including a schedule of usable animals, examples of postoperative care records, examples of observation records on animals used to test treatment drugs, and articles on research in which the anesthetic protocol included the same anesthetic as that used as the principal anesthetic in the LSU research. Bibliographic information for these articles is provided in appendix VIII.

The veterinary anesthesiologists we consulted reviewed the same information that we provided to the medical panel for the June meeting. They also reviewed the additional information we obtained during our meetings with LSU in September and November 1989.

In April 1990, we also provided each member of the medical panel much of the additional information obtained from LSU. This report incorporates their comments as appropriate.

We did our review between January and December 1989, in accordance with generally accepted government auditing standards.

[6]Michael E. Carey and others, "Experimental Missile Wound to the Brain," Journal of Neurosurgery, Vol. 71 (1989), p. 754.

Research Has Merit but Validity of Results Questioned

We convened a panel of medical experts in June 1989 to review the research. These panelists reviewed the contract proposals and various reports sent to the Army under contract requirements. The experts provided their individual comments to questions that GAO had asked (see app. V), followed several months later with a summary of the individual comments and the panel's discussion compiled by the panel chairman (see app. VI). The panel was generally positive about the research except for one panel member who was generally less supportive of the project than the others. The panel believes that this type of research is the only way progress can be achieved in treatment, that there is no current research in this area, and that the model is unique. The panel considers the principal investigator a highly respected member of the neurosurgical community with long-standing interest in missile injury and unique clinical experience in the battlefield. Although most panelists expressed concerns about research performance in some areas, the panel concluded that the project had merit and funding should continue.

The panel also concluded, on the basis of the university's AAALAC accreditation, that the care of the animals at LSU has been adequate. The chief consultant on the panel for the care of animals believes that LSU has more than adequately met the AAALAC standards. The panel did not believe that it could specifically evaluate the adequacy of postoperative care from the documentation reviewed. But the panel believes that the anesthetics used throughout the research were adequate to protect the animals from pain during wounding. The panel also noted that the brain has no nerve endings per se and does not suffer pain postoperatively.

Because the areas in which most of the panelists expressed concerns could affect some research results, we reviewed these areas further. The majority of the members of the panel were concerned about management of the anesthesia and postoperative care.

We consulted five veterinary anesthesiologists on issues specifically related to these areas. We also asked them to comment on any other aspects of research performance they believed to be important. Their analysis of the research raised questions about the validity of some of the research results.

All general anesthetics affect cerebral metabolism, blood flow, and the brain's ability to autoregulate (regulate its own blood flow). The degree of alteration and the mechanism by which it occurs vary depending on the anesthetic. Therefore, the anesthetic can have significant implications for some research results unless it is precisely controlled. The veterinary anesthesiologists concluded that the information they reviewed indicated inadequate control of the anesthetic; as a result, the anesthetic's effects on some research results are unclear.

Postoperative care also affects some results. All aspects of the recovery from anesthesia and postoperative care should be detailed to confirm that uniform care was provided to all animals. The veterinary anesthesiologists pointed out that careful management of the postoperative period (that is, monitoring such factors as body temperature, fluid balance, and reflexes) is important in comparing the recovery of treated animals with untreated ones to identify effective drug treatments. In general, LSU did not maintain postoperative care records. The veterinary anesthesiologists indicated that they could not determine the adequacy of postoperative care from the information provided to them.

The veterinary anesthesiologists raised questions about other aspects of research performance. For example, the three anesthesiologists who commented on blood gas data believe that errors may have occurred in blood gas measurements. Two of the three also questioned whether the trauma model provided "graded" responses, that is, whether it provided different responses to injuries of increasing severity. Four veterinary anesthesiologists had concerns about the project's experimental failure rate,[1] which was more than 2-1/2 times greater than LSU estimated; all of the anesthesiologists questioned the differences between the number of animals used and the number of animals for which data were reported.

Questions About Control of General Anesthesia

The primary issue in the management of a general anesthetic is proper control, which requires maintaining a uniform depth of anesthesia so data can be compared within and across groups. The anesthetic and its method of administration in the research made controlling the depth of anesthesia difficult.[2] Anesthesia was induced using pentobarbital, administered intraperitoneally (IP) in the abdomen and maintained through bolus (all at once) intravenous (IV) injections of pentobarbital. Barbiturates such as pentobarbital (which were introduced in 1930 and

[1]Failure rate refers to the percentage of animals used in the experiment that did not produce usable (reportable) data.

[2]Throughout the research period, pentobarbital was the principal general anesthetic. Other anesthetics, such as halothane and isoflurane, were used infrequently. Although some animals used early in the research initially received IV pentobarbital, primarily it has been administered IP.

are widely used in research) produce dose-dependent responses in physiological parameters critical to the outcome of the LSU research (that is, cerebral blood flow and cerebral metabolism);[3] these barbiturates will affect the results unless the dose is precisely controlled. The veterinary anesthesiologists' review of the research reports raised questions about whether control of the anesthetic was adequate.

Management of Anesthetic Difficult

Cats metabolize (eliminate) pentobarbital more slowly than many other animals and, thus, have a prolonged recovery period from its effects.[4] The respiratory function of animals, especially the cat, is particularly sensitive to the effects of barbiturates. Doses of barbiturates (such as pentobarbital) that induce deep anesthesia, sufficient for surgical intervention without a response to pain, severely depress both the respiratory frequency and tidal volume (volume of breath). This results in dangerously low levels of oxygen in the blood as well as body tissues and increased levels of carbon dioxide; this increase produces an imbalance in the oxygen-carbon dioxide levels in the blood. Depending on the overall condition of individual cats and doses administered, the resulting depressed ventilation may cause the animal to stop breathing and die.

The veterinary anesthesiologists agreed that the difficulty in controlling the depth of anesthesia is compounded when it is administered by IP injection, which prolongs absorption of the anesthetic; this results in a slow induction and inconsistent depth of anesthesia, as well as a prolonged recovery from its effects. Unless the individual administering the drug is well trained, it is easy to inject an overdose; if this occurs, the drug cannot be quickly eliminated or detoxified. The intermittent administration of IV boluses of pentobarbital anesthesia does not produce a

[3]Barbiturates, derivatives of barbituric acid, are used as hypnotic and sedative drugs. Modifications in their structure influence the potency and rapidity of their effects. The depressant effects of these drugs are exerted on the higher centers of the brain.

[4]"At one time pentobarbital was the principal IV anesthetic used in veterinary medicine. However, safer procedures using techniques of balanced anesthesia [the use of an additive combination of drugs to produce general anesthesia; therefore, the dose of a single drug reduces the side effects of the second drug] have essentially replaced pentobarbital in modern practice. Further, the depth or level of anesthesia is less readily controlled with drugs injected intravenously or parenterally, whereas it is easily controlled with volatile drugs like ether, halothane, and methoxyflurane . . . In general, most laboratory animals metabolize drugs more rapidly than humans. The cat, however, is an exception and requires a longer time to metabolize barbiturates. The cat shows a marked susceptibility of respiratory function following barbiturate administration. Barbiturates must be induced with particular caution." In Nicholas H. Booth and Leslie E. McDonald, eds., Veterinary Pharmacology and Therapeutics, 5th ed. (Ames, Iowa: Iowa State University Press, 1982), pps. 206 and 211.

consistent level of anesthesia, nor does it compensate or equalize the initial IP administration. The IV boluses only create varying depths of anesthesia after a variable initial dose, making comparison difficult.

Anesthesia Dose May Not Have Been Regulated

The veterinary anesthesiologists indicated that it is extremely important that the general anesthesia be administered in a careful and controlled manner so that the reactions of study and control animals can be compared. In this way, any changes that occur will be the result of the trauma rather than the anesthetic. Many anesthesiologists have found, one veterinary anesthesiologist explained, that (1) brain disease, tumors, and trauma modify brain function and blood flow and (2) the effects of anesthetic agents are unpredictable. Therefore, he continued, in any cerebral trauma model it is important that the depth of anesthesia be precisely controlled so that any changes that occur—which can be due to changes in cerebral autoregulation, blood flow, or metabolism—are due to the damage and not to changes in carbon dioxide, anesthetic levels, or body temperature.

In the documentation they reviewed, the veterinary anesthesiologists saw no evidence that the dose of anesthesia was precisely regulated so that the depth of anesthesia was controlled. Further, because the anesthetic was administered IP and maintained with bolus IV injections, the depth of anesthesia and the duration could vary during and between experiments.

For the most part, anesthesia records were not kept on individual animals used in the experiments. LSU did not believe such records were important; it indicated that although specific anesthesia data generally were not recorded, protocols applicable to the 33 experiments were followed (see app. III for protocols). We found that when records were kept, however, doses actually given varied significantly and do not agree with the protocols.

Our review of the laboratory notebooks found anesthesia records detailing cat weight, anesthesia doses, and times of administration for only about 20 to 25 percent of the animals used in the experiments. These records show that the anesthesia protocols were not followed. For example, the protocol states that the initial anesthesia dose is 40 milligrams per kilogram of animal weight of IP-administered pentobarbital. We compared this protocol with the doses in the records and found that the initial dose of anesthetic actually given ranged from 14.2 to 61.9 milligrams per kilogram of animal weight.

Inhalant anesthetics would be preferable, three of the veterinary anesthesiologists commented, but pentobarbital could be used as a general anesthetic for the LSU research if the conditions of its administration were precisely controlled. They emphasized, however, that this precludes IP administration. They added that if pentobarbital is used, the preferred method is IV administration so as to achieve a steady state level of anesthesia by continuous infusion. One veterinary anesthesiologist stated that to

> . . . establish a constant infusion of a barbiturate to achieve a steady-state level during the study, [it is necessary to] control ventilation and maintain the carbon dioxide tensions [levels] between 38 and 42 mm/Hg [millimeters of mercury], and maintain the oxygen tension [level] of 95-100 mm/Hg. An oxygen tension of 95-100 mm/Hg is consistent with oxygen tensions when breathing room air and a carbon dioxide tension of 38-42 mm/Hg is consistent with the awake state. Body temperatures should be maintained between 37 and 38° Centigrade.

On the basis of the information they reviewed, the three veterinary anesthesiologists who addressed this issue do not believe that the research achieved these levels of control.

Questions About Effect and Adequacy of Postoperative Care

For animals allowed to awaken from anesthesia, careful monitoring of postoperative care is important to obtain data relevant to research objectives and help ensure appropriate recovery. However, the research team did not consider postoperative care factors important to the research and did not adequately document the postoperative care given to the animals. As a result, the effects or adequacy of the postoperative care on research results can not be determined.

Effects of Postoperative Care Not Considered

The veterinary anesthesiologists emphasized that all aspects of postoperative care should be documented in detail to confirm that uniform treatment was provided to all animals. In contrast, the research team stated that it does not believe that postoperative care, which occurs after the 6-hour experimental period, is relevant to the research design or analysis. However, as reported in LSU's final report on the first contract (see figs. IX.1, IX.2, and IX.3 in app. IX), results on brain water, sodium, and potassium levels are presented for animals up to 7 days postwounding.

Without monitoring of physiologic parameters and observations of behavior, research data cannot be accurately interpreted. Monitoring assures appropriate analysis when comparing anesthetized animals (the effects of pentobarbital alone) with injured untreated animals (the effects of the missile wound on the brain) and injured treated animals (the effects of drug treatment on the missile wound) with other groups of animals. One veterinary anesthesiologist specified that postoperative monitoring should include tracking, treating any possible pain exhibited by the animal, and documenting observations. These physiologic parameters, he added, are especially important for head trauma studies, in which changes in reflexes and behavior—such as slow return of reflexes, excitement, reduced body temperature, or unconsciousness—can be expected after wounding. These changes may also result from the anesthesia.

Records Not Maintained

In general, records were not maintained for the postoperative care given to animals recovering from the experimental period. The proposal for the first contract mentions only that animals were to be observed over a 6-hour period and then sacrificed while still under anesthesia. This protocol was later modified as the Army approved a request to extend the life of the animals postoperatively so that brain swelling and other abnormal reactions occurring outside the 6-hour period could be observed.

The second contract contained the following paragraph describing the postoperative care plan for a 4-month period:

> Surgery and wound closure will be sterile. We will wound these cats with a sterile sphere to obviate infection. After wounding and closure of the 4 centimeter scalp wound, we will remove chronic cats from the stereotaxic frame so they will have no pain upon awakening. We will remove the endotracheal tube as soon as possible. These wounded animals will be placed in warmed cages in our laboratory for intensive nursing care as needed during their early post-wounding convalescence. Maintenance fluids will be by IP route. We will treat them with penicillin and apply local antibiotic to the wounds.[5]

A retrospective description of postoperative care, given to animals used under the first contract, was provided in the Journal of Neurosurgery:

> Cats allowed to recover from anesthesia and wounding were treated with local antibiotic ointment and topical anesthetic to all sutured skin incisions. They were given Penicillin G (50,000 units, intramuscularly [IM]), carefully nursed, and observed in the animal care facility until they had fully recovered. Normal saline

[5]This plan was reviewed and approved by the LSU Animal Care and Use Committee 3 months after the second contract was awarded to LSU by the Army.

solution was given intraperitoneal [IP] for the first few days after wounding, if necessary, for hydration. During the recovery period no cat appeared in any pain.[6]

Additionally, postoperative care for the animals used in the first contract was provided in the Journal of Neurotrauma:

Cats allowed to live beyond 6 hours were wounded with an alcohol-soaked pellet wrapped in sterile paper before it was inserted into the gun barrel. These animals were also given penicillin (300,000-450,000 units i.m.) after wounding. Local antibiotics and topical lidocaine were also applied to all wounds that had been sutured closed. No cats allowed to recover from anesthesia appeared in any pain prior to sacrifice. The animals were painlessly sacrificed by barbiturate overdose and exsanguination from 6 hours to 7 days after wounding.[7]

Questions About Postoperative Pain

We discussed with the veterinary anesthesiologists the issue of postoperative pain. Two anesthesiologists noted that the wound itself would not cause pain since the brain has no nerve endings. Yet three anesthesiologists believe that the animals would experience pain from (1) the incisions made to insert various catheters and monitors and to remove the anterior wall of the right frontal sinus and (2) any swelling that might result from the injury. Four anesthesiologists stated that the animals used in the research would require postoperative analgesics. One anesthesiologist commented that a topical anesthetic ointment is insufficient for pain relief since the ointment has poor tissue penetration and provides relief for only 6 to 7 minutes.

During our visit to LSU, we interviewed the veterinarian who has cared for the brain-wounded animals in the LSU animal care facility. Individual records detailing the postoperative care and recovery for each animal were not maintained. However, the veterinarian told us that the animals from the brain-wound project experienced pain. He also told us that he treated them for the pain with butorphanol tartrate, an analgesic drug. In addition, he stated, the animals receive fluids by subcutaneous injection, but are not force-fed or supported through any other nutritional means.

We recounted the meeting with the LSU veterinarian in discussions with research team members. They indicated that they were unaware that

[6]Michael E. Carey and others, "Experiment Missile Wound to the Brain," Journal of Neurosurgery, Vol. 71 (Nov. 1989), p. 754.

[7]Michael E. Carey and others, "Brain Edema Following an Experimental Missile Wound to the Brain," Journal of Neurotrauma, Vol. 7, no. 1 (Spring 1990), pp. 13-20.

the animals received analgesics, saying that they had not ordered any and would object to their use. However, the team did not believe anything that might have been done in the animal care facility had affected the research results. In a meeting with LSU officials 2 months later, we received a signed statement from the LSU veterinarian stating that he had given analgesics to only one animal.

Questions About Other Aspects of Research Performance

Lack of detail in other aspects of the research performance raised additional questions from the veterinary anesthesiologists about the validity of the reported results. The veterinary anesthesiologists' review of the research data, as reported to the Army, suggests that (1) there are possible blood gas measurement errors; (2) the trauma model is not a predictable "graded-response" model (producing different responses to injuries of increasing severity); and (3) the trauma model has had an unusually high failure rate. Further, data on all animals used in each experiment have not been reported.

Blood Gas Data Questionable

Blood gas concentrations are one measure of the depth of anesthesia. The three veterinary anesthesiologists who commented on the blood gas experiments indicated that LSU's reported data on oxygen and carbon dioxide levels (see table 2.1) suggest measurement errors and that the LSU researchers were unable to control blood gases. These veterinary anesthesiologists believe that the incongruities in the blood gas data, unless explained, may invalidate related research results.

The LSU report to the Army, interpreting data in table 2.1 (see also table IX.1), states

> that brain wounding may exert a profound influence upon "central" [medullary] respiratory drive mechanisms. Additionally, we have monitored arterial blood gases after wounding in 15 cats and have determined that brain wounding also may be associated with significant "peripheral" [pulmonary] effects as well: hypoxia [too little oxygen], hypercarbia [too much carbon dioxide], and acidosis [too much acid]. While often these effects accrue from the apnea itself, sometimes they are not the result of decreased central respiratory drive mechanisms.

Table 2.1: Research Data on Arterial Blood Gases

		Prewounding			1 minute postwounding		
Wound energy	Cat no.	Resp. rate	pO_2	pCO_2	Resp. rate	pO_2	pCO_2
0.9J[a]	219	18	100.2	37.8	0	121.8	26.5
0.9J	227	14	81.2	31.8	8	63.7	35.7
0.9J[b]	231	8	82.7	46.8	10	65.7	50.4
0.9J	233	12	82.6	42.0	0	59.8	39.7
0.9J	239	16	102.9	40.8	20	121.7	39.9
1.4J	225	20	101.6	29.9	0	59.4	41.4
1.4J	228	24	74.3	40.7	19	71.7	41.9
1.4J	234	8	109.8	38.0	0	39.3	46.9
1.4J	237	14	113.6	40.9	0	46.8	50.9
1.4J[b]	243	10	111.4	42.3	14	61.2	51.9
2.4J	220	12	60.8	32.7	12	47.1	31.5
2.4J	223	12	127.5	44.0	6	120.0	36.6
2.4J	236	13	91.5	43.5	8	51.5	48.7
2.4J	241	12	105.8	44.6	0	57.9	50.3
2.4J[b]	244	16	120.6	40.1	21	72.9	50.9

[a]J refers to joules.

[b]Animals exhibiting significant decreased arterial pO_2, hypercarbia, and decreased pH without central respiratory depression.

Source: Selected data taken from table 10 (p. 47) of LSU's final report on the first contract submitted to the Army February 10, 1987. (See table IX.1 for the complete table.)

The three veterinary anesthesiologists pointed out several incongruities in these data. Included in the table are blood gas measurements for prewounding (that is, the animals were anesthetized, but unwounded) and postwounding. But for prewounding, the animals are only under the influence of the anesthesia. Data are not, therefore, within expected ranges for these animals, even after allowing for differences in individual animals.

One veterinary anesthesiologist explained that for cats breathing room air (20 percent oxygen), the normal oxygen (pO_2) and carbon dioxide (pCO_2) tensions (levels) are 95 to 100 millimeters of mercury (mm/Hg) and 38 to 42 mm/Hg, respectively. The blood gas levels should be maintained at these levels for cats under anesthesia. However, as shown in table 2.1, prewounding resulted in 14 of 15 animals with an oxygen level outside the normal range and 7 of 15 animals with a carbon dioxide level outside the normal.

Another veterinary anesthesiologist commented that the combined carbon dioxide and oxygen values for several of the animals are not possible as reported. He indicated that values (for a cat breathing room air at sea level) below 120 mm/Hg indicate poor lung function; values above 160 mm/Hg indicate laboratory error (120 to 130 mm/Hg and 150 to 160 mm/Hg are gray zones). Totals for cats #239, #223, and #244—162, 172, and 161 mm/Hg, respectively—exceed the upper range of possible values.

This veterinary anesthesiologist also commented that as shown by the data in table 2.1, the magnitude of change for the carbon dioxide (pCO_2) values 1 minute postwounding are greater than expected. He said that raised the question of whether the measurements were actually documented at 1 minute. A carbon dioxide (pCO_2) increase of about 2 mm/Hg per minute would be expected in an animal that is not breathing. However, cats #227, #231, #225, #234, #237, #243, #236, #241, and #244 experienced carbon dioxide increases of 3.9, 3.6, 11.5, 8.9, 10.0, 9.6, 5.2, 5.7, and 10.8 mm/Hg, respectively, at 1 minute postwounding.

Questions About the Trauma Model

The Army awarded the contracts on the assumption that a valid model existed for studying the pathophysiology of fragment injuries to the brain and testing various treatment drugs. Two veterinary anesthesiologists commented that the trauma model lacks different responses to injuries of increasing severity. In addition, three veterinary anesthesiologists commented on the high failure rate of the model.

Model Does Not Produce Graded Responses

Reports to the Army state that the trauma model has been developed based on a "faithful" replication of fragment wounds to the brain inflicted at three levels of increasing severity—0.9 joules, 1.4 joules, and 2.4 joules. A graded-response model, such as this one, should demonstrate progressive and statistically different responses for injuries inflicted at different levels of energy (missile impact). However, two of the veterinary anesthesiologists who examined these data concluded that the model does not predictably produce graded responses. For example, in one experiment, the reported data indicate that wounded animals experienced an increase in intracranial pressure (ICP) over control animals. But the data (see tables IX.2-IX.5) show no evidence that there are significant differences between the 0.9-, 1.4-, and 2.4-joule degrees of trauma. This is also the case for data reporting the effects of wounds on amounts of substances in the brain, including water, sodium, and potassium in white matter. (See figs. IX.1-IX.3.)

Another example of incomplete reporting comes from the cerebral blood flow experiment, discussed earlier in this report (see p. 31). That experiment used a total of 45 animals; 4 animals died prematurely, reducing the total usable animals to 41. Of this number, 13 were control animals and 28 were study animals. The first annual report on the second contract includes data from 5 (versus 13) control animals and 23 (versus 28) study animals. The remaining 13 cats are not accounted for. However, since the report never states that a total of 45 animals was used, the discrepancy is not apparent.

Two veterinary anesthesiologists commented that there are no generally accepted criteria for reporting data. They generally believe data reporting is an ethical issue. All five anesthesiologists believe that given the current scientific climate—minimizing the use of experimental animals for both humane and cost purposes—there is a marked disproportion between reported and unreported animals in this project. One anesthesiologist commented that it is difficult to have confidence in the reported results when so many animals have been excluded with no explanation as to the effect that data for them would have on the results.

table 2.2.) The 1986 and 1988 experimental groups were further divided into two groups each, on the basis of the general anesthetic used—pentobarbital or isoflurane. In the first contract, animals anesthetized with pentobarbital were not fully awake until 1 or 2 days postwounding. Given the behavioral scale used at the time to assess recovery of neurologic function and to test drug efficacy, the period for testing drugs was short; therefore, the shorter-acting isoflurane was also used. In the 1989 experimental group, all of the animals were anesthetized with pentobarbital. As shown in the table, the death rate was generally much higher than LSU expected.

Table 2.2: Death Rate by Type of General Anesthesia

	Pentobarbital			Isoflurane		
	Number			Number		
Experimental year	Wounded	Died	Percent	Wounded	Died	Percent
1986	3	1	33	6	1	16
1988	10	5	50	6	5	83
1989	27	12	40	[a]	[a]	[a]

Note: All animals used in this experiment were wounded at the .9-joule level (the lowest energy level used to inflict the brain injury). No animals were wounded in 1987 for use in testing drug treatment therapies.

[a]Isoflurane was not used in 1989.

Not All Data Are Reported

The reported results do not discuss data from experimental failures. Our comparison of the laboratory notebooks with reports submitted to the Army showed substantial differences between the number of animals used and the number for which data are reported.

During our visit to LSU in September 1989, we met with the research team to further discuss the methodology used in each of the 33 experiments and to review the laboratory notebooks to determine the number of animals used. In tracing the animals used in each of the experiments to the reports on results, we found that data from a large number of animals were not included in the reports. For example, we identified the experiments included in the November 1989 Journal of Neurosurgery article and reviewed the information in the laboratory notebooks on the animals used in those experiments. We found that the data came from three experiments (listed in app. III as "Electrolytes," "Physiology," and "Apnea"), which used a total of 165 animals, but the article refers to 103 animals. No mention is made of the remaining 62 animals used in the experiments for which the results are reported.

In addition, one of the veterinary anesthesiologists commented that the reported data from an experiment on cerebral blood flow also raise questions about whether the model produces graded responses. (See tables IX.6 and IX.7.) Animals used in that experiment were wounded at different energy levels and were differentiated by the presence or absence of blood clots in the brain postwounding. However, data for injuries inflicted at different energy levels were aggregated for reporting.

A High Failure Rate for Model

The second LSU proposal estimates the number of animals needed for the research and plans for a "failure rate" of about 14 percent; that is, about 14 percent of the animals used in the experiments were not expected to provide usable data. The average actual failure rate has been about 2-1/2 times greater than that estimated. Data LSU provided us in November 1989 show the disposition of 648 cats used in the project's 33 experiments (see app. X). On the basis of these data, the failure rate averaged 37 percent, ranging from about 14 percent to 61 percent.[8]

Two veterinary anesthesiologists commented that there are no generally accepted criteria for establishing the failure rate of a model. They stated that the rate depends on the nature of trauma and can be higher in the early stages of model development. The four anesthesiologists who commented on the failure rate of the LSU experiments said, however, that the rate was unacceptably high, especially for animals that were not wounded. Two of these four anesthesiologists commented that it is an indication that (1) a predictable model was not developed; (2) too many variables were uncontrolled; or (3) the response to head trauma is so variable that a progressive model cannot be produced.

In some experiments, the ratio of animals providing usable data to total animals used did not seem to improve over time. For example, one experiment—referred to by LSU as a "behavioral" experiment—was critical to the drug-testing objective of both contracts. All of the animals in this experiment were wounded at the 0.9-joule level (the lowest energy level used to inflict the brain injury) because the animals needed to survive so they could be used for long-term drug testing. This experiment used a total of 52 cats in three groups, 1986, 1988, and 1989. (See

[8]The data list the following as unusable: 3 "pilot study" animals, 4 "assay check animals," 6 "technique development" animals, and 9 "unsuccessful initial experiment" animals. Eliminating these 22 animals—which may be considered as part of the model development—reduces the failure rate to about 34 percent. The reasons why these animals were excluded include "died after wounding," "massive brain bleeding," "ICP outside acceptable levels," "overnight deaths," and "physiological instability prewound."

Army's Management of the Contracts Inadequate

The Army's contracts with LSU for brain wound research do not violate the law limiting the use of dogs and cats in DOD projects. However, the Army's management of the contracts has been inadequate. A number of significant changes were made to the research scope and methodology specified in the research objectives and contract requirements; in most cases, the Army was not notified of these changes. They could have been detected from progress reports submitted to the Army, but contract file documentation does not indicate such an effort. Moreover, progress reports frequently were not filed within the periods required by the contracts.

In general, the Army has appeared to take little notice of the research performance; the Army has also made no apparent effort to ensure that the work specified in the contracts was performed or was performed in such a way that it would contribute to the body of knowledge about treating brain wounds. Despite detailed operating procedures delineating contract-monitoring responsibilities, the contracts have been poorly monitored and technical assistance has not been provided when it would have appeared to be appropriate.

Contracts Do Not Violate Public Law on DOD Use of Cats and Dogs

A portion of the funds for the current LSU contract with the Army was provided by DOD's Appropriations Act for Fiscal Year 1988 (P.L. 100-202). Section 8056 of this law contains the following provision:

> None of the funds appropriated by this Act shall be used to purchase dogs or cats or otherwise fund the use of dogs or cats for the purpose of training Department of Defense students or other personnel in surgical or other medical treatment of wounds produced by any type of weapon. . . .

Because the LSU contracts are research efforts and not training, the use of cats in this project does not violate this law.

The language of the provision has been included in the general provisions section of DOD appropriations acts every year since fiscal year 1984.

Contract Performance Poorly Monitored

The Army contract-monitoring procedures—for example, site visits and review of progress reports—provide the means for determining whether the research activities are consistent with contract requirements and ensure that the results of the research will be of value. The COR has the primary responsibility for (1) ensuring that a contract's technical objectives are met and (2) guiding and evaluating the research performance.

During the course of this research project, assignment of CORs has lacked continuity. At times, the contract has not had a COR. Site visits have been made infrequently; contractually required progress reports have often been submitted late, combined with other reports, or not submitted at all. COR technical review of reports was not thorough.

COR Has Primary Responsibility for Monitoring Technical Aspects of Research Performance

The Army's contracting officer stated that the Army uses contracts, rather than grants, as the method of acquiring research because contracts provide for more control over performance. Contract specialists assist in the legal and financial aspects of contract monitoring. Additionally, the Contracting Officer's Representative (COR) is appointed to monitor the technical and scientific aspects of the contract. The COR manages through site visits and reviews of contractor progress reports.

Although contracts facilitate the Army's control over its research projects, they still allow for contractor flexibility since agreed-on research objectives may be changed by notifying the Army and obtaining its approval. The contract requires quarterly and annual progress reports, as well as a final report at the conclusion of the research project. The Army has the authority to redirect the research effort or to terminate the effort at the convenience of the government.

The technical monitoring responsibilities of the COR are contained in the Army's Acquisition Guide for Contracting Officer's Representatives and Program Officers and include

- directing the contractor to redirect the contract effort or shift work emphasis between work areas or tasks within the scope of the work,
- providing information to the contractor concerning the work,
- reviewing and approving the required reports from the contractor, and
- conducting animal care and use inspections to ascertain compliance with federal regulatory requirements.

The Guide also suggests that a dialogue be maintained between the contractor and the COR. According to the Army's contracting officer, there is no set number of monitoring visits for a research project, although 1 visit per year is the goal.

At Times No COR Monitored the Contractor's Performance

Although the COR has a critical role in monitoring the technical aspects of research performance, there has been no COR for a significant portion of the time the LSU contracts have been in effect. Since the first LSU contract became effective in 1983, four different CORs have been appointed to monitor the contracts. However, for two periods (one 6 months; another, 5 months), no COR was responsible for monitoring. Further, for a 19-month period in the second contract, a contract specialist monitored legal and financial aspects as well as technical and scientific aspects without the guidance or assistance of a scientist or doctor. Thus, for 30 of the total 71 months or about 42 percent of the time the contracts had been in effect, only a contract specialist was assigned to monitor both the legal and scientific aspects of contract performance.

Further, the lack of continuity in CORs might have disrupted the consistency and level of the monitoring, oversight activities. Army officials explained that the difficulty in maintaining COR continuity stems from the mission-driven reassignment of personnel.

COR's Monitoring Limited and Lacked Depth

Since the research began, the Army's COR for this contract made infrequent site visits to the project. Further, the reports prepared on these visits do not indicate any follow up of concerns noted in the reports.

The first visit to the LSU brain wound laboratory was made by the Army's animal use review officer, not by the COR. The visit was made on July 20, 1984, about 1 year into the performance period of the first contract. The trip report indicated that after initial delays, the contractor proceeded to develop a wounding technique that produced a predictable wound and was making progress toward the overall research objectives.

The first visit made by a COR was on February 21, 1986—2 months before the performance period of the second contract was to begin and about 8 months after the Army sent the research proposal, which resulted in the second contract, for peer review. The trip report, although general, was supportive of the project. For example, in the report, the COR stated that the project was very productive and cost-effective up to that point because three manuscripts for publication were nearing completion, with two more possibly to follow. The report also indicated, however, concerns with the model. There was no indication in the report or other documentation in the contract file that the Army took any action on these concerns.

The second, and last, site visit by a COR was made on June 5, 1987—about 14 months into the second contract. The trip report indicated that the performance appeared to be in full compliance with the terms of the contract and that no deviation from the stated research objectives was expected. However, before the COR's visit, in June 1987, experiments were conducted that were not included in contract objectives. For example, laboratory notebooks show that from May 27 to June 17, 1987, experiments were completed on four animals to "perfect a cerebral ventricular cannula for chronic measurement of ICP and CSF [cerebrospinal fluid] sampling."

The COR's review of the first contract's final report and the last annual report also suggest that appropriate attention was not given to determining whether (1) the research as performed followed the required scope of work or (2) there were any reasons for not following the contract requirements. For example, testing treatment drugs was an objective of the first contract, yet the principal investigator stated that no drugs were tested.

The Army officials maintain that CORs have a variety of demands made on their time in addition to those basic to the position. The current COR stated that he tries to "keep on top of the [LSU] contract," but that he has several other contracts for which he is responsible, supervises a 30-person division, and is running his own intramural research projects at Letterman Army Institute of Research.

This COR also stated that he relies on the reports submitted by LSU and expects LSU to call him if contract problems occur. He stated that because of his other duties, he has visited the LSU laboratory only twice; in addition, on occasion, he calls LSU, but he maintains no records or telephone logs that indicate the frequency of these contacts or the subject matter discussed.

Required Reporting Frequently Late

The majority of the required contract reports (quarterly and annual progress reports and the final report on the first contract) have been submitted to the Army late. Except for one reference in the trip report, filed after a site visit to the laboratory, little evidence indicates that the Army has attempted to enforce its reporting provisions.

Language in the second contract underscores the importance of the quarterly reports as the most immediate and direct contact between the contractor and the COR. The annual report is to include these parts: a complete, clear summary of the previous year's research activities; a comprehensive data presentation to provide a complete, accurate record of research findings; a summary of statistical tests used and level of significance obtained; the number of observations for averaged data; and all experimental methods used during the reporting period, referenced to formal publications or presented in enough detail so that another investigator, working from the annual report, could repeat the experiments.

The final report is to be a summary report covering the entire term of the contract. The report must present an interpretation of the data and findings of the completed project, including explanations for unexpected results or those that do not fit within the working hypothesis.

According to the terms of the two contracts, as of October 15, 1989, 23 quarterly reports, 4 annual reports, and 1 final report should have been submitted to the Army.[1] However, 3 of the 23 quarterly reports were not submitted; 18 of the remaining 20 (90 percent) were submitted late, ranging from 2 days to 5 months late. One annual report was submitted in combination with the final report on the first contract. The remaining annual report, covering the period from April 1988 to April 1989, had not been received as of October 1, 1989. The final report for the first contract was 13.5 months late; by the time it was submitted, work on the second contract had been under way for 10 months.

Project Changes Recommended by the Army's Peer Review Panel Not Made

LSU did not make changes to the research scope and methodology recommended by the Army peer review panel evaluating the proposal for the second contract. The Army stated that LSU was not required to respond to Army peer review comments because they were intended for internal Army use in ranking the scientific merit of all proposals submitted for possible funding.

The minutes of the Army peer review panel meeting, during which the LSU proposal (among others) was evaluated, stated:

> The investigator needs to address two problems before finalizing the protocol. First the enormous size and complexity of the project needs to be reduced. They have

[1] The first contract states that (1) quarterly reports are to be submitted within 15 days after the quarter ends; (2) annual reports are to be submitted concurrently with the annual renewal request; and (3) the final report, within 90 days after contract expiration. (The second contract states that quarterly reports are due on the date the quarter ends; annual reports are to be submitted within 30 days of the end of the reporting period.)

> proposed that a total of 220 cats be purchased and used during the first year. The many different experimental groups would seem to reduce the size of any single group to a fairly small number. For example, one of the experimental groups is planned to consist of only six cats in each of the normotensive [normal blood pressure] and hypotensive [low blood pressure resulting from major loss of blood through hemorrhage] study groups. The measurements they intend to make can be expected to be extremely variable, and a group of six animals would be likely to produce scattered data with very little clear interpretation possible. It seems that this is a bit of a fishing expedition, and while such an approach to an important problem may in fact be worthwhile, one should give oneself the best opportunity to derive meaningful data. The project might benefit from fewer studies with more animals in each group in order to produce more consistent data.
>
> The second problem is the proposed use of the Wiggers model for hypovolemic [low blood volume] shock. This introduces unnecessary complications by reinfusing the shed blood, which can be expected to contain all of the mediator substances that are activated in the animal during hypovolemia. This seems to add an unnecessary complication to the already complex model. . . .In fact, the use of hypotensive and normotensive animals is a compounding of the problems, and [it] seems that the initial approach might be simplified even further by studying only normotensive animals without all of the potential artifacts of the hypovolemic model.

During our visit to LSU in February 1989 to discuss the research project, LSU acknowledged that the Army communicated these concerns to them. LSU did not make the changes, however, because the research team did not believe it was necessary.

Contractor Made Scope and Methodology Changes Without Obtaining the Army's Approval

The panel commented that many of the changes made in the scope and methodology were to be expected because changes in research protocols are often made in the course of a research project—after the project starts and problems are encountered. The panel believes that these changes improved the research effort. We noted, however, that some methodological changes were made in areas that experts had raised questions about, such as changes in general anesthesia. Further, many of these changes were made without getting prior written approval—as contractually required—from the Army.

As to change procedures, both contracts state that

> Written approval of the contracting officer shall be obtained prior to change of the methodology or experiment, stated objectives of the research effort, or the phenomenon or phenomena under study.

For the first contract, the Army approved LSU requests for modifications in the project's time, costs, and objectives (deletion of BBB work). However, other changes to objectives were made without the written approval of the contracting officer. For example, the principal anesthetic for the research was changed with no documented approval. Although the research proposal indicated that halothane, an inhalant, would be used as the principal general anesthetic, it was replaced with pentobarbital, a difficult anesthetic to control. This substitution was not documented until the first contract's final report, dated February 10, 1987. Similarly, throughout the performance of the second contract, pentobarbital was used predominantly rather than the inhalants indicated in the proposal.

Technical Assistance Not Provided When It Might Have Been Appropriate

The contract-monitoring procedures give the Army the opportunity to provide technical assistance, to guide and direct aspects of the research, and to participate in decision making during the project; these procedures increase the probability of a successful project. The Army provided technical assistance early in the research effort, when the researchers experienced difficulties with the gun. Assistance was not provided at other times, however, when it appeared to be appropriate to direct or participate in decision making to help resolve performance-related issues. For example, changes to the anesthetic protocols the Army reviewed and approved would appear to have warranted at least an inquiry from the COR about the reason for the change and how this change might affect research results, if at all.

The earliest reported indication that LSU changed the anesthetic came after work on the first contract was completed; however, an inquiry would have still been relevant to determine its impact on the second contract. This report (submitted 10 months into the performance period of the second contract) also indicated that the trauma model has limitations for drug testing because of the small time period between recovery from anesthesia and the point in time when wounded cats appear perfectly normal—3 to 4 days. Since drug testing was also an objective of the second contract, notice of the trauma model's limitations is a reasonable basis for inquiry and assistance from the Army.

Conclusions, Recommendations, Agency Comments, and Our Evaluation

Conclusions

The Army entered into two consecutive research contracts with LSU: (1) to study the pathophysiology of brain wounds and (2) to develop a drug treatment protocol that would be effective in treating soldiers on the battlefield who are brain injured by shell and other fragments. These soldiers could then be returned to duty, thereby conserving military fighting strength. The LSU research effort has been under way since 1983.

The medical panel that met at GAO, in June 1989, concluded that research in this area is important because (1) no one else is working in this particular area and (2) the research model is unique. Although most panel members raised some concerns about the performance of the research in several areas, the panel concluded that the project had merit and funding should continue. Given that the areas about which panelists expressed concerns—management of general anesthesia and postoperative care—could affect some aspects of the research results, GAO reviewed these areas further.

The veterinary anesthesiologists we consulted had several concerns that raised doubts about the validity of some of the research results.[1] Their specific concerns included

- lack of anesthesia and postoperative-care records for individual animals used,
- imprecise control of the anesthesia,
- inappropriate method of administering the anesthetic,
- no consideration of how postoperative care affects results,
- no postoperative analgesics to assure optimal pain relief for experimental animals,
- incongruities in reported blood gas data,
- the trauma model's lack of different responses to injuries of increasing severity,
- the trauma model's high failure rate, and
- discrepancies between the number of reported and unreported animals used in experiments.

We believe that these concerns, taken together, suggest the need for careful assessment of the project's future.

[1]One concern was about the particular anesthetic, pentobarbital, which was used in the research. It has essentially been replaced as an anesthetic in veterinary medicine by inhalants.

We do not know whether the concerns discussed above would have been identified and resolved earlier in the contract period if the Army had properly managed the technical performance of the LSU research. We found, however, that the Army's management of the contracts has been inadequate. The Army did not enforce the provisions of the contract with LSU, follow Army procedures for monitoring the performance of the research, or provide technical assistance when appropriate.

Recommendations to the Secretary of Defense

We recommend first that the Secretary decide if the project benefits have been substantially achieved already. If so, the Secretary should not continue the project.

If the Secretary finds that the benefits have not been substantially achieved, we recommend that he review the concerns raised in this report to determine if continuing the contract will produce additional useful information. If, after this review, the Secretary finds it desirable to continue the project, then we further recommend that he ensure that the concerns we identified have been resolved.

DOD and LSU Comments

DOD and LSU provided written comments on a draft of this report. DOD partially agreed with our findings for the Army's management and monitoring of the LSU contract; DOD has taken corrective actions. In addition, DOD concurred with our recommendations for DOD procedures relating to decisions on whether to continue funding of the LSU project on brain-wound research. DOD has scheduled reviews and assessments of the brain-wound research to implement these recommendations.

DOD and LSU disagreed with our observations on scientific issues related to

- control of general anesthesia and its potential effect on some research results;
- the effect and adequacy of postoperative care; and
- other aspects of research performance including questions about (1) the possible recording of measurement errors in blood gas values, (2) the ability of the trauma model to produce predictable graded responses, (3) failure rates during the performance of the project, and (4) concerns about data-reporting methods.

LSU also disagreed, in part, with the process we used to conduct our review.

We believe that both the process used to conduct our review and the concerns raised are valid. Members of our medical panel expressed concern that poor anesthetic and postoperative management could modify or skew some research results. After panel members raised these concerns, we consulted veterinary anesthesiologists, a specialty not represented on our panel, to explore these concerns further. Professional differences of opinion exist on these scientific issues. Our recommendations are intended to focus DOD's attention on these issues as it decides whether to continue the LSU project.

The following is a summary of the DOD and LSU comments along with our response. The full text of the DOD and LSU comments are presented in appendices XI and XII respectively.

Conduct of Our Review

LSU (see app. XII) raised concerns about the conduct of our review and our use of the comments from the medical panel. Our review, LSU said, did not allow an exchange of information between qualified scientists and did not permit the medical panel to visit the LSU laboratory. LSU also said the medical panel worked solely from information selected from the laboratory by us.

It was not our intention to model our study on peer review processes, such as those used at the National Institutes of Health or other grant-giving organizations. We provided our panel members the same information available to the Army when it peer reviewed the contract proposals and monitored contract performance on the basis of reports received from LSU. In conducting a study, we typically use multiple approaches, such as engaging consultants and collecting our own data, as appropriate. It is often difficult to reach consensus among experts with diverse backgrounds, especially when addressing a broad range of highly technical issues. In the final analysis, we have responsibility for both the study approach and the conclusions drawn.

In our review of the LSU project, we did not "independently" (see app. XII) select information to provide to our medical panel from the laboratory notebooks, staff, or any other source. The only information we provided to the panel consisted of (1) the first and second contract proposals, (2) the final report on the first contract, (3) one of two required annual reports for the first contract, (4) two of the three required annual reports for the second contract, and (5) three of the four required quarterly reports for the third year of the second contract. LSU had not submitted the missing annual and quarterly reports, as required by the contract, to the Army. We provided the proposals and reports to the panel and did not conduct independent analyses of the documents as input for the panel's deliberations.

In addition, we provided the panel with information on the anesthesia used in the experiments, the number of experiments performed, and the number of animals used in each experiment. This information was gathered, at our request, by the LSU research staff and reviewed by the principal investigator. We did not interpret this information in any way and simply photocopied the data that LSU typed and provided to us. For example, LSU states that we incorrectly reported that the principal investigator performed 33 types of experiments when his data clearly indicated that there were only 9 "areas of research interest" (see app. XII). However, it was LSU's organization and categorization of the data into 33 types of experiments that we reported. (See app. III for the LSU research team's descriptions, in June, of the experiments, with updated information from the team in September.)

We did prepare a list of questions to guide the review and requested each panel member's comments on these questions. The questions, along with the individual responses of panel members, appear in appendix V.

Control of General Anesthesia

DOD and LSU disagreed with our assertions that the LSU studies lacked proper dose control of the anesthesia and, therefore, raised doubts about the validity of some of the research results. Our report was modified to recognize that injections of pentobarbital into the animals' abdominal cavities were accompanied by IV injections as needed (see pp. 5, 22, and 23). We maintain our position, however, that we saw no evidence in the documentation that the anesthesia was precisely regulated and in accordance with established protocols for the project (see app. III, pp. 187-190 and 206-207 for statement of the protocols).

Members of our medical panel expressed concerns about the potential effects of the anesthetic agent and the method of administration. The panel members did not specifically outline the deficiencies of the anesthetic management, but expressed concerns that poor anesthetic and postoperative management could modify or skew some research results. The role of the veterinary anesthesiologists, a specialty not represented on the panel, was to explore these concerns further. Our board-certified veterinary anesthesiologists are preeminently qualified to judge matters pertaining to anesthetic methods, postoperative care, and general presentation of data.

Effect and Adequacy of Postoperative Care

DOD stated that "All cats used in the Louisiana State University study were terminal, either they died as a result of the study or were euthanized for histopathological examination." We disagree. LSU states in its comments that about 13 percent of all cats used in the experiments were allowed to survive. Our records indicate that about 33 percent of the animals lived from 24 hours to several years after the 6-hour experimental period. In addition, LSU's final report on the first contract presents results on brain water, sodium, and potassium levels for animals up to 7 days postwounding (see figures IX.1, IX.2, and IX.3 in app. IX). Consequently, we also disagree with the DOD position that postoperative care was not relevant to the research design and analysis.

Although LSU maintains that animals were monitored, our reviewers wanted to know who monitored the animals and how frequently. Given the absence of detailed records of the actual care provided the animals who survived the 6-hour experimental period, our concerns about postoperative care remain unanswered. We believe the burden of proof rests with the principal investigator to show that research results have not been skewed by inconsistent or undocumented postoperative treatment.

Questions About Other Aspects of Research Performance

DOD and LSU disagreed with our observations about other aspects of research performance that raised questions about the validity of some of the reported results. Specifically, DOD and LSU disagreed with observations pertaining to (1) possible errors in blood gas measurements, (2) whether the LSU model produces predictable graded responses, (3) the high failure rate of the trauma model, and (4) concerns about data-reporting methods.

After reviewing the additional information provided, our primary veterinary anesthesiologist believes that the explanations are not sufficient to alleviate concerns about the potential effect of our observations on the reported results. The importance of these differences of opinion is a matter of interpretation and, therefore, our recommendations refer these issues for consideration and resolution by the Secretary of Defense.

Army Monitoring of Contract Performance

DOD partially agreed with our observations about shortcomings in the Army's monitoring of contract performance. DOD noted that although the Army contract system encourages the COR to conduct annual site visits, most problems that arise on contracts can be handled by telephone. DOD has taken steps to make sure future telephone contacts are fully documented. DOD and LSU also agreed that the principal investigator frequently missed required reporting dates. According to DOD, an enforcement mechanism has been established that requires the contract specialist to return all vouchers unpaid to the contractor if required reports have not been submitted on time.

DOD said that LSU was not required to respond to the Army peer review comments because they were intended for internal use by the Army in ranking the scientific merit of all proposals submitted for funding. DOD stated that subsequent contract changes by LSU did not constitute changes in methodologies, stated objectives of research effort, or the phenomena under study. However, DOD agreed that revisions, such as changes in general anesthesia, should have been discussed with the Army before implementation. DOD has advised research investigators, for the future, to follow such procedures before making contract changes.

Adequacy of Technical Assistance

DOD partially agreed with our observation that technical assistance has not been provided by the Army when it might have been appropriate. DOD reached this position because it cannot verify the level of technical assistance actually provided given the lack of documentation.

Proposal I

LSU's first proposal resulted in a contract, "The Effects of an Experimental Missile Wound to the Brain on Brain Electrolytes, Regional Cerebral Blood Flow and Blood Brain Barrier Permeability; The Treatment of the Resultant Disorders." This contract began on July 1, 1983, and ended on December 31, 1985. The following is the complete proposal, except for the "Detailed Budget" and the "Budget Justification", which were deleted by the Department of the Army. Personal information on the principal investigator was deleted by us.

The Effect of an Experimental Missile Wound to the Brain on Brain Electrolytes, Regional Cerebral Blood Flow and Blood Brain Barrier Permeability; The Treatment of the Resultant Disorders

Starting Date - March 1983

Duration of Support - 3 years

Principal Investigator: Michael E. Carey, MD

Professor of Neurosurgery

Department of Neurosurgery

LSU Medical Center

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New Orleans, LA 70112

(504) 568-6123

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Michael E Carey MD
Principal Investigator

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Department of Neurosurgery

January 24, 1983

*Commander, Letterman Army Institute
of Research
ATTN: SGRD-ULZ-RCM/B. McHenry
Presidio of San Francisco
California 94129*

Dear Mrs. McHenry:

Thank you for your recent phone calls concerning my missile wound project.

The animal care area at LSU is staffed by two full time veterinarians (Drs. Gonzales and Longoria) who are fully capable of diagnosing feline diseases (and all laboratory animal diseases). Each began practice before Veterinary Boards were instituted and each has approximately 20 years laboratory animal experience. The animal quarters are AAALAC approved. The quarters are air conditioned and maintained at 72-75°F, ambient humidity. Only one cat will be housed in each cat cage. The dimensions of the cages are 4 square feet (floor) x 24 inches high. They will be fed Purina cat chow and the cages will be cleaned daily by animal care personnel. They will be sanitized every other week. Any desired day-night cycle can be requested and we will use 12 hours light-12 hour dark. I have read the brochure "Guide for the care and use of laboratory animal " DHEW (NIH) 78-23, 1978. Animals will be housed and treated according to these precepts.

The cat has been widely used in experiments on brain electrolytes and brain edema[1,2,3,4,5]. Cats have also been used for microsphere, blood flow experiments[6,7,8,9,10]. The most recent ballistics experiments in the literature have used monkeys (grant refs 19-26 p 32-33) but I feel that this is far too expensive a model. Monkeys will not provide significantly better brain data than cats. Irsigler[11] at the Kaiser Wilhelm Institute in Berlin studied ballistic brain lesions in cats quite successfully.

School of Allied Health Professions | School of Graduate Studies | School of Medicine in Shreveport
School of Dentistry | School of Medicine in New Orleans | School of Nursing

1. Graziani L J, Escriva A: Calcium exchange between brain and blood in cats and immature and adult rats Neurology 19:314-315, 1969

2. Bradbury MWB, Kleeman CR, Bagdoyan H, Bergerian A: The calcium and magnesium content of skeletal muscle, brain and CSF as determined by atomic absorption flame photometry J Lab Clin Med 71:884-892, 1968

3. Pappius HM, Oh JH, Dossetor JB: The effects of rapid hemodialysis on brain tissues and CSF. Canad J Physiol Phamacol 45:129-147, 1967

4. Pappius HM: Effects of steroids on cold injury edema. in Reulen HJ, Schurmann K (eds) Steroids and Brain Edema, New York, Springer-Verlag 1972, p 57

5. Long DM, Maxwell RE, Choi KS et al: Multiple Therapeutic Approaches in the treatment of brain edema induced by a standard cold lesion. in Reulen HJ, Schurmann K (eds) Steroid and Brain Edema, New York, Springer-Verlag 1972 p 87

6. Alm A, Bill A: The oxygen supply to the retina. 11 Effects of high intraocular pressure and increased CO_2 tension on uveal and retinal blood flow in cats. A study with labelled microspheres including flow determinations in the brain and other tissues
Acta Physiologica Scand 84:306-319, 1972

7. ibid: The effect of stimulation of the cervical sympathetic chain on retinal oxygen tension and uveal retinal and cerebral blood flow in cats.
Acta Physiologica Scand 88:84-94 1973

8. Fara JW, Madden K: Effect of secretin and cholecystokinin on small intestinal blood flow distribution.
Am J Physiol 229:1365-1370, 1975

9. Nissen 01, Galskov A: Direct measurement of superficial and deep venous flow in the cat kidney.
Circ Res 30: 82-96, 1972

10. Reneman RS et al: Vertebral and carotid blood distribution in the brain of the dog and cat. Cardiovascular Research 8:65-72 1974

11. Irsigler FJ: The healing process of experimental brain wounds in the case of open and closed brain lacunae. Zentr fur Neurochir 7: 1-43, 1942

(2)

I hope this provides the information you wish.

Sincerely,

Michael E. Carey

Michael E. Carey, M.D.
Professor of Neurosurgery
L.S.U. Medical Center

MEC:eah

1. Graziani L J, Escriva A: Calcium exchange between brain and blood in cats and immature and adult rats Neurology 19:314-315, 1969

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Principal Investigator

School Official

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BIOGRAPHICAL SKETCH OF PRINCIPAL INVESTIGATOR

Michael E. Carey, MD 042-28-4206

Pertinant personal and educational background are provided in the CV.

I began neurosurgical practice in Hartford, Connecticut in 1967. Drafted in 1968, I served as Chief of Neurosurgery at the 312th-91st Evacuation Hospital in Chu Lai, RVN, Sep 1968-Aug 1969. While there I kept precise records on our patients and have published 9 clinical papers relative to war wounds (see CV). I have data for several more clinical neurosurgical papers relative to RVN. I have reviewed the WDMET head wound data (at Edgewood Arsenal) and presented some of this at the 4th International Ballistics Symposium, Gothenberg, Sweden, 1981.

I have continued in the Active Army Reserve to the present and because of my interest in war neurosurgery I am writing the official neurosurgical history of the Vietnam War for the Surgeon General, US Army, under the auspices of the US Army Historical Department.

Following active duty in the Army I joined the neurosurgical department at Louisiana State University School of Medicine in New Orleans. I have developed a very active laboratory interest in physiology. After publishing several papers in cerebrospinal fluid physiology, during 1978-1979 I took a sabbatical year in London, England to further study physiology with Professors Hugh Davson and Michael Bradbury at King's College. There, I learned many important physiologic concepts, strengthened my math abilities and learned how to work with radioisotopes and measure brain electrolytes. Currently in the laboratory I am measuring 3 H_2O PS products in rats using [14C] iodoantipyrine as the cerebral blood flow marker (the Ohno technique to be used during the 03 year of this project).

I am an actively practicing, Board Certified neurosurgeon working with neurosurgical residents at LSU and treating private patients. This busy schedule will require a full-time on-site PhD (to be named) and a research associate to make the project go hour by hour, day by day. I plan to be at LSUSM-NO for the duration of this project.

SUMMARY

In combat the head receives about 20% of all "hits." Forty percent of all deaths are from brain wounds. Neurosurgical mortality of combat-incurred brain wounds was 14% US, WWII; 9.6% US, Korea; and 10-12% US, Vietnam. These data indicate no reduction in brain wound mortality for US forces over the past 35 years.

Despite the extreme importance of brain wounds as a major source of combat mortality (both nonoperative and postsurgical) and the importance of such wounds for long term post-wounding morbidity and disability, I can find less than a dozen papers published during the last decade on experimental missile wounds to the brain. These papers by and large have concentrated on the brain-missile wound's effect on peripheral phenomena : peripheral vasculature hemodynamics, blood pressure, cardiac output, and respirations. Crockard studied many of these phenomena as well as intracranial pressure, cerebral blood flow and brainstem evoked responses following a missile wound to the brain in monkeys. Those experiments, however, measured only gross, hemispheral blood flows. No other specific physiological functions of the brain were directly studied. Data from these experiments implicated brainstem dysfunction after a missile wound yet no direct physiologic measurements of brainstem function were made. Most seriously, however, gross hemispheral blood flows may not reflect blood flow in the critical brainstem areas at all.

In my proposed project I will create a standardized, experimental, nonfatal missile wound in cats to study how a missile wound to the brain interferes with several of the more important physiologic phenomena associated with normal brain function. I will study brain electrolytes; regional cerebral blood flow (CBF) and CBF autoregulation and blood brain barrier (BBB) permeability. These physiologic functions are important to study because brain electrolytes reflect BBB and cellular integrity. The brain cannot function unless its blood supply is intact and normal BBB permeability provides one of the brain's chief homeostatic mechanisms.

Dexamethasone, mannitol, and dimethylsulfoxide (DMSO) have all been proposed for the treatment of brain wounds. Despite the widespread use of dexamethasone in Vietnam for brain wounds there are no experimental data on its efficacy following a missile wound. Likewise, experimental data are lacking which show a beneficial effect of mannitol or DMSO on the brain. I will evaluate the effect of these drugs on brain electrolytes, CBF, and BBB permeability following a missile wound to the brain.

Because current mortality associated with brain wounds is so high, better treatment for those sustaining a brain wound is one of the remaining ways whereby a major reduction in combat mortality can be achieved. Better treatment can only come from a more sophisticated understanding of the pathophysiology associated with the brain missile wound. Decreasing the mortality and neurologic morbidity associated with head wounds will result in substantial financial savings to the US Army and Federal Government. Ascertaining appropriate drugs with which to treat brain wounds may simplify Army purchasing needs and reduce costs.

BACKGROUND

Past Statistical Data

In modern wars the head receives about 16 to 27% of all "hits"[1,2] and head wounds account for about 40% of all combat deaths.[3,4] During WWI the postoperative neurosurgical mortality was at least 31%.[5,6] In WWII postoperative neurosurgical mortality for the Allies ranged from 10 to 17%,[5,7,8] whereas in Korea, it was 9.6%.[9] Neurosurgical mortality for brain wounds incurred in Vietnam was 10-12%.[10,11] These data for U.S. forces indicate that there has been no decrease in the overall lethality of head wounds and no significant decrease in combat neurosurgical mortality from 1945 to 1975.

Past Research on Missile Wounds to the Brain

Despite these facts, little basic research on brain wounds has been done. Through WWII, research concentrated on the "explosive" effects of fatal, high-velocity missiles[12-15] though Webster and Gurdjian,[16] studied intracranial pressure (ICP), blood pressure (BP), respiration, and mortality in dogs following a brain missile wound. Recently, Gerber[17] restudied the hemodynamic effects of a missile wound to the brain while Djördjevic[18] produced severe, fatal brain missile wounds in dogs. He attributed high ICP after wounding to intracranial bleeding (despite the fact that little free blood was found in the cranial cavity) and an arterial pressor response.

Crockard[19-26] has undertaken the most comprehensive, recent studies on brain missile wounds by creating nonfatal missile wounds in adult rhesus monkeys. He found good correlation between missile energy and physiological effects. After brain wounding, the respiratory pattern changed but arterial blood gases (ABGs) did not. The mean blood pressure (MBP) fell, then rose, whereas the ICP rose, then fell. The cerebral perfusion pressure (CPP) fell about 50% (CPP = MBP-ICP, mm Hg). Cerebral vascular resistance (CVR) increased, and concomittantly cerebral blood flow (CBF) decreased. Cerebral blood flow autoregulation failed. These phenomena were attributed to direct brainstem effects of the missile altering brainstem vasomotor efferents. Interestingly, cardiac output (CO) was also depressed. Treatment of the brain-wounded monkey with mannitol or dimethylsulfoxide (DMSO) reduced ICP and increased MBP, CPP, and CBF. These drugs were believed to exert their beneficial effects by increasing CO.

Qualifications of Past Research Efforts

Although Crockard's studies admirably brought some modern physiological techniques to bear on the problem of brain wounding, it must be noted that he inflicted the missile wound through a trephine opening in the skull. Any physiological effects produced by the shock wave of a missile first striking bone (as in real life) could not have been observed. More important, however, CBF was measured by 133 Xenon wash out and external counting, which can only indicate gross, hemispheral CBFs. These may bear no relation to CBF in smaller vital brain areas as the brainstem. Furthermore, CVRs calculated from hemispheral CBF may be totally unrelated to CVRs in critical brainstem structures. Because brainstem dysfunction appears to be so important in Crockard's data, specific knowledge of physiologic function there is important: perhaps brainstem dysfunction following missile wounding results from ischemia (vasomotor paralysis)[27] or transient blood-brain barrier (BBB) opening.

Reduction in CO consequent to brain missile wounding appears to be an important phenomenon, but Crockard measured CO in only 8 monkeys. Verification of this physiologic effect, perhaps as part of a generalized trauma response,[23] should be attempted in other animals. If it is a constant finding, further delineation and treatment of reduced CO may indeed lead to improved brain function in those sustaining brain missile wounds.

Finally, Crockard's recent studies only measured three actual brain-related parameters: ICP, hemispheric blood flow and evoked potentials. Many additional and important physiologic measures of brain function should be studied following a missile wound because proper and advanced treatment must be designed to minimize physiologic abnormalities in the brain itself.

Other Physiologic Parameters Bearing on Normal Brain Function

1. Brain Water, Brain Electrolytes:

The brain is approximately 80% water,[28] and normally brain sodium (Na) is ~ 55mEq/liter while brain potassium (K) is ~85mEq/liter (Na/K = 0.65). Normal brain ion concentrations depend on normal cellular membrane function and an intact BBB. BBB disruption produces vasogenic brain edema (VBE). Transient or permanent BBB disruption allows plasma albumin, sodium, or other osmotically active molecules to enter the brain extracellular space (ECS). Water passively follows the passage of these molecules[29] and the water content of the affected brain increases.[28,30] Simultaneously, the Na/K ratio increases as brain Na rises and brain K falls.[28,30,31]

Several authors[31-33] have reported that dexamethasone decreases VBE after a standard, experimental cold lesion. Others, however, have failed to demonstrate beneficial effects of steroids on cerebral edema.[28,34,35] From Vietnam, Hammon[10] reported a 10% mortality for brain wounds where dexamethasone was routinely used; Carey,[11] on the other hand, did not use steroids in the treatment of brain wounds and reported 12% mortality. Thus, both experimentally and clinically, the effect of steroids on traumatic brain edema is not totally resolved. I can find no experimental studies on the effect of steroids on brain edema or brain electrolytes after a missile wound.

Crockard found both mannitol and DMSO improved brain function by increasing CO. He did not measure possible direct effects of these therapeutic measures on the brain. Long[36] showed that DMSO decreased brain water following a freeze lesion but did not measure brain electrolytes. We will investigate the actions of dexamethasone, mannitol and DMSO on brain water and electrolytes after a missile wound.

2. The Blood Brain Barrier

The BBB provides one of the brain's chief homeostatic, protective mechanisms.[37] Its permeability has been studied physiologically by perfusing the brain vascular space with a graded seried of nontransported, nonmetabolized polar non-electrolytes. Smaller molecular weight (MW) molecules, as aminoisobutyric acid (MW 103.1), have smaller diameters than do larger molecules, as sucrose (MW 342), polyethylene glycol (MW 400-4000), or serum albumin (MW 58,000) and therefore pass the BBB more readily. The ease with which such test molecules diffuse across the BBB (a measure of BBB permeability) can be quantified by their so-called permeability-surface area (PS) products.[37]

$$PS = -CBF\ Vc\ \ln(1-E) \qquad (1)$$

P	=	permeability coefficient
S	=	surface area of capillaries
CBF	=	cerebral blood flow
Vc	=	volume in which the test molecule is dissolved during the experiment
E	=	the extraction fraction (see equation 7, p 14)

Alterations of BBB permeability with trauma[39,40] or other means[41] has often been demonstrated by the leakage of larger molecules, such as Evans-Blue or iodine-labelled serum albumin[40,42] from the vascular compartment into the brain ECS. [14C] sucrose has been used in nontraumatic experimental models to demonstrate transient BBB openings.[43]

Obviously, missile injury to the brain will disrupt the BBB around the missile track. Use of serum albumin to estimate BBB permeability will document only the grossest BBB disruptions. Smaller BBB openings at a distance from the actual wound can only be documented by using smaller test moledecules and quantified by expressing BBB permeability mathematically (as by PS). A systematic examination of BBB porosity consequent to missile wounding is important because leakage of small vasoactive amines,[44] such as norepinephrine through the BBB could cause regional CBF changes in critical areas, such the brainstem. Brainstem ischemia could conceivably explain many of Crockard's findings.

After cold injury, apparently steroids decrease BBB permeability, as measured by radioactive serum albumin.[40,42] Whether steroids alter BBB permeability measured by other, smaller test molecules is unknown. Furthermore, whether treatment with steroids affects the BBB at all after a missile wound is also unknown. Despite this fact, steroids were widely used in Vietnam.[10] Crockard found that mannitol and DMSO improved CO and brain function. Whether these drugs have a more direct effect on the missile-wounded brain is unknown. We will study their effect on BBB permeability after a missile wound.

3. Regional Blood Flow

Both Djördjevic[18] and Crockard[2] studied gross but not regional cerebral hemisphere CBF after a missile wound by 133 Xenon washout. Regional CBF studies would be more informative to more fully understand brain missile wounding because within the closed skull, energy may be transmitted at a great distance from the actual missile track. This transmitted energy may cause vascular-ischemic effects throughout the brain.

We will measure regional CBFs in the brain after a missile wound both by microspheres and [14C] iodoantipyrine. Crockard's data suggest that reduced CO consequent to the brain missile wound lies behind much of the observed brain dysfunction. CO can be measured with radioactive microspheres (reference syringe withdrawal method) and CO, in turn, is used to calculate regional CBF.[47-55] The microsphere technique, therefore, is particularly appropriate for our intended experiments.

The Autoregulation of CBF

CBF autoregulation (constant brain blood flow despite falling BP, within limits) is an inherent, protective property of the normal brain vascular system. Classically, CBF autoregulation has been tested by hemorrhagic hypotension. CBF autoregulation is very important to the combat soldier because, when wounded, severe blood loss and hypotension are common. CBF autoregulation tends to prevent brain ischemia in these circumstances. More than 50% of those who receive brain wounds in combat also receive other wounds and often concomittant hypotension occurs. No experimental data exist regarding CBF autoregulation following a missile wound though it is known to be lost following other trauma. Crockard inferred that a dysfunction of cerebral autoregulation occurred after a brain missile wound but never specifically tested for it by measuring CBF following BP reduction.

Drugs which might improve blood flow to the brain

After a cold lesion, steroids improve brain function beyond their ability to reduce edema.[46] Whether dexamethasone improves brain electrolytes or BBB permeability after a missile wound will be studied in these experiments and any improvement will be correlated with regional CBF changes or CO enhancement. Crockard noted that mannitol and DMSO improved CO. He did not ascertain whether these treatments improved regional CBFs. Improvement in brainstem perfusion, for instance, might have superseded increased CO. To my knowledge no data exist on whether drugs may enhance impaired CBF autoregulation after a missile wound.

HYPOTHESIS

A nonfatal missile wound to the brain will cause several important physiologic disruptions, among them: BBB permeability increases, vasogenic brain edema, brain electrolyte alterations and regional cerebral blood flow changes. Alterations of these physiologic functions will occur not only adjacent to the missile track but also at a distance from the wound, possibly in the brainstem. Such distant alterations may explain observed brainstem effects[19-28] associated with a brain wound.

OBJECTIVES

(A) To document the acute changes in (1) brain water and electrolytes; (2) regional cerebral blood flow and cardiac output; and (3) BBB permeability consequent to a nonfatal missile wound in cats.

(B) To see whether the use of dexamethasone, mannitol, or DMSO given 1 hour after wounding minimizes or prevents physiological dysfunction of these 5 variables. In our experiments treatment will commence one hour after wounding because, in combat, treatment probably would not occur before this time. Though barbiturates have been shown to provide some protection for the ischemic brain,[56] they will not be included in this study because drowsiness associated with their use would probably render them impractical in a combat environment.

METHOD

The Missile: We will use the lightest mass (m) test missile possible, a 0.030 gm steel sphere fired from a special air gun. The sphere will traverse 2 electronic gates to determine its velocity (v) and allow its kinetic energy (KE) to be calculated: ($KE = 1/2\ mv^2$). Preliminary experiments will be done to select an appropriate v such that the missile will traverse both frontral lobes of the cat rostral to the lateral ventricles but not exit the brain. Current ballistic theory considers that the Energy of Deposit (E_D) causes tissue damage. $E_D = KE_{(entrance)} - KE_{(exit)}$. If a missile does not exit, $KE_{(exit)} = 0$ and $E_D = KE_{(entrance)}$. The wound will not be fatal. Relative to total brain weight, a 0.030 gm missile in a cat is somewhat larger than most missiles which cause brain wounds in humans. This scaling factor will be considered in data interpretation.

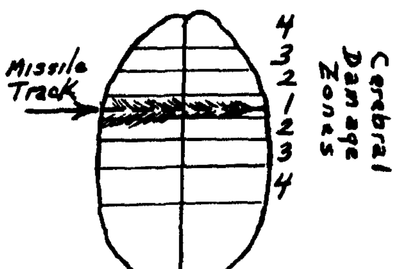
Animals: Cats will be used because they have ample white matter, are small, and will not require large and expensive radioisotope doses. They are relatively inexpensive.

General Preparation: (including preparation for light microscopy—brain water and electrolytes) We will place unselected, nonfasting cats initially in a closed chamber connected to an anesthesia machine.[A] Anesthesia will be induced with 3% halothane[B]-oxygen and maintained on 0.5% halothane. We will insert 2 PE 90[C] femoral artery catheters, one for BP recordings (precalibrated (RP 1500) transducer[D] and physiograph[E]) and the other for hematocrit and arterial blood gas (ABG) determinations. We will insert a PE 90 catheter into a femoral vein for saline and drug administration. We will tracheostomize the cats, tie in an endotracheal tube, shave the head and swab all wounds with local anesthetic (Nupercaine[F]). We will cast[G] the hind legs to protect the catheters. We will use a rectal Hg thermometer to measure temperature, kept at $37 \pm 1°C$ by a heating blanket.

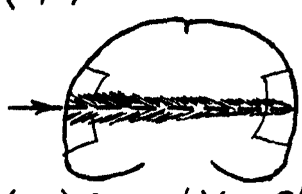
After surgery, we will tranquilize the cat with phencyclidine 1-2 mg/kg[H] and stop the halothane because it alters CBF and CBF autoregulation.[58,59] Two hours later, we will briefly anesthetize the cats with methohexital sodium[60] 30 mg/kg i.v., place them in a stereotaxic frame,[I] and connect the tracheostomy tube to a cat respirator[J]-anesthesia machine. We will maintain anesthesia with N_2O/O_2::70/30, keeping arterial pCO_2 ~35 mmHg, pO_2 ~80 mmHg, and pH 7.40. ABGs will be measured by ILmicro 313/326.[K] One hour after methohexital administration, we will anesthetize (1% xylocaine)[L] the left side of the scalp through which the missile will enter the skull. The brain will then be shot with a 30 mg steel sphere. Immediately thereafter, we will place anesthetic ointment in the skin wound, remove the cat from the stereotaxic frame, and discontinue the N_2O. Phencyclidine, however, will be continued as needed.[19] Post wounding BPs and ABGs will be monitored in all cats.

01 YEAR: Light Microscopy: We will sacrifice experimental cats at these post-wounding times: 10 sec, 10 min, 30 min, 1 hr, 2 hr, 3 hr, and 6 hr, three cats for each period. Two control cats will be sacrificed immediately after halothane induction; two controls will be prepared as experimental cats and sacrificed at 6 hours. (Total Cats: 25) Ten minutes before sacrifice, 1 ml of 2% Evans blue[M] will be given i.v. to all cats. We will give each animal 30 mg/kg methohexital[N] i.v. 5 sec before sacrifice and then exsanguinate the cat. The total brain will be quickly removed and suspended by its basilar artery in 10% formalin. After adequate fixation, we will section the midbrain separating the cerebral hemispheres and adjacent structures from the brainstem-cerebellum. All sections will be paraffin embedded. The hemispheres will be sliced horizontally in 10μ cuts. Every 50th slice will be stained (H and E, cresyl violet, or Bodian). The brainstem and cerebellum will be sectioned coronally and similarly sectioned and stained. Appropriate photographs to demonstrate gross damage will be taken. We will document all histologic changes microscopically. The "standard" missile track within the brain will thus be delineated; adjacent and distant histologic alterations will be documented, both grossly and microscopically.

Histologic criteria will be used to establish four "zones" of cerebral hemisphere injury at increasing distance from the missile track (Fig. 1). We will devise a measuring system to ensure that all subsequent cerebral hemisphere samples in all further experiments will come from these four demarcated zones. All tissue samples will be centered on the plane of the missile track (Fig. 2). The following brain areas will be delineated and obtained in all subsequent experiments:



(Fig 1) Top View Brain



(Fig 2) Coronal View Showing Brain Samples In Relation to Plane of Missile Track

Table 1. Brain Samples

Cerebral	Hemisphere	Right	Left	Total Samples
	Zone			
	1 (Track)	1 gray, 1 white	1 gray, 1 white	4
	2	1 gray, 1 white	1 gray, 1 white	4
	3	1 gray, 1 white	1 gray, 1 white	4
	4	1 gray, 1 white	1 gray, 1 white	4
Basal Ganglia		1 Right	1 Left	2
Thalamus		1 Right	1 Left	2
Mesencephalon		1 Right	1 Left	2
Pons		1 Right	1 Left	2
Medulla		1 Right	1 Left	2
Cerebellum		1 Right Hemisphere	1 Left Hemisphere	2
Total				28

01 YEAR: Brain Water and Electrolytes: After wounding, cats for brain water and electrolyte analysis will receive an i.v. bolus of methohexital and be sacrificed by exsanguination at these times: 10 sec, 10 min, 30 min, 1 hr, 2 hr, 3 hr, and 6 hr. We will use five cats for each experimental period. (35 Cats) Control cats will be sacrificed after anesthesia but without surgical preparation and at these times after surgical preparations similar to that of experimental animals: 30 min, 60 min, 2 hr, 3 hr, and 6 hr. If anesthesia and surgical preparations cause brain water and electrolyte changes to occur in controls, we will need 30 controls (5 for each period). If brain water electrolytes remain stable, we will need only 10 controls (5 without surgery; 5 at 6 hr after surgical preparation).

Brain Water and Electrolytes Following Brain Wounding and Treatment:

One hour after wounding experimental cats will receive i.v. one of the following drugs: either dexamethasone[O] 5.0 mg/kg;[61] mannitol[P] 0.5 gm/kg (25% solution)[25] or DMSO[Q] 0.5 gm/kg (50% solution)[26] q 1 hr until sacrifice. They will be sacrificed 2 and 5 hr after treatment. We will use 5 cats at each time period for each drug (30 experimental cats). Controls will be prepared as experimentals but not shot. They will receive the drugs starting one hour after being placed in the stereotaxic frame and then sacrificed 2 and 5 hr later. Maximally we will need 30 controls. If possible we will combine control data and use fewer cats.

Handling of Brains for Brain Water and Electrolytes:

Immediately after sacrifice (see p. 9) the entire brain will be removed, placed in a humidity chamber and dissected to obtain tissue samples per Table 1. 50-100 mg brain samples will be placed in washed, deionized (0.75 N HNO_3) low Na glass, tared pots which will be immediately covered and weighed. The samples will then be uncovered placed in an oven,[R] and dried to a constant weight for 48 hr at 100°C. Following this, the sample pots will be recovered, placed in a dessicator, cooled and reweighed. Brain water = wet weight - dry weight; tissue swelling = $\frac{\Delta H_2O\% \ (100\%)}{DW\%}$. ($\Delta H_2O$ = the difference in % water content of control and experimental tissue and DW% = the dry weight in % of the edematous tissue).[62]

Brain char will then be ground and a known volume of 0.75N HNO_3 added to each pot. Brain electrolytes will be leached for 48 hours. The mixture will be centrifuged and Na^+, K^+ and Cl^- determined on the supernatant (IL 443;[R] Buchler Chloridometer[AA]).

02 YEAR: Regional CBF and CBF Autoregulation Following a Missile Wound to the Brain Determined by Microspheres

Anesthetic induction and maintenance, cannula insertion, tracheostomy, and stereotaxic frame placement will be as in General Preparation (p 7). In addition a brachial artery catheter will be placed for BP recording. One femoral artery catheter will be advanced retrograde into the left ventricle of the heart for microsphere injection (and hematocrit and ABGs). Proximal catheter tip position will be ascertained by BP tracing and will be checked post mortem. The other femoral artery catheter will be attached to a saline-filled 5 ml syringe held in a constant withdrawal pump[S] (reference blood withdrawal). ICP will be measured by an epidural, 5 mm Happenstein balloon[19] placed through a 6 mm right posterior trephine. The skull defect will be sealed with dental acrylic.[T] The saline filled epidural catheter will be attached to a pressure transducer[U] set at earbar level. We will measure ICPs with each CBF measurement.

Microsphere Preparation and Injection

We will use 3M[V] 15 $\pm$ 3μ diam. carbonized microspheres (Cerium 141, [145 KEV]; Strontium 85 [514 KEV] and Scandium 46 [890 and 1120 KEV]). Each isotopic microsphere (MS) injection allows one CBF to be made. We will inject $\sim$ 1.0 x 10^6 microspheres (MS) for each CBF measurement, making a maximum of 3 injections and CBF determinations per cat. We will process each MS aliquot as follows:[63,64] We will vortex the 3M MS bottle (0.1 mCi contained in 45 x 10^6 MS in 10 ml 10% dextran, 0.05% Tween), withdraw 1 x 10^6 MS in a sterile tuberculin syringe and place this in a plastic counting vial. We will bring the volume to 2.0 ml with normal saline (NS). The vial (or a vial aliquot) will be counted on a gamma counter to determine "precounts". We will sonicate the MS-containing vial for 30 min, then vortex it, and remove 2 drops of fluid to microscopically check for MS dispersion. The residual MS containing fluid in the vial will be withdrawn into a new, sterile 3.0 ml syringe. The now empty counting vial will be recapped and counted. The MS-containing syringe will be continually agitated and attached to the femoral catheter going to the heart. We will start the constant speed, femoral artery blood withdrawal pump, and then inject the microspheres over 15-20 seconds, monitoring heart rate and BP concomittantly. We will then clamp the injection catheter and flush it with 1-2 ml of N.S. Following injection we will count the empty injection syringe as well as the withdrawn blood. After the experiment both the infusion and withdrawal catheters will be counted as well. Any spillage from the injection syringe will be caught on Kleenex and this Kleenex also counted.

$$\text{Counts Injected} = \text{"Pre Counts"} - \text{Vial Residual Counts} - \text{Residual Counts Syringe} - \text{Residual Counts Injection Catheter} - \text{Spillage} \quad (2)$$

$$\text{CO} = \text{Counts Injected} \times \frac{\text{reference syringe withdrawal rate}}{\text{counts in blood} + \text{counts in withdrawal cannula}} \quad (3)$$

$$\text{rCBF} = \text{CO} \times \frac{\text{Isotope Counts in Brain Region}}{\text{Isotope Counts Injected}} \quad (4)$$

Following final injection we will remove the cat's brains, harden them in dry iced Freon 12 and sample brain areas designated in Table 1. Brain samples will be placed in tared counting vials. The vials will be reweighed and counted in a 3 channel Beckman[W] 8600 gamma counter for which a program has been written.

In preliminary experiments we will adjust injected counts so that cardiovascular effects are avoided with injection, each brain area has> 400 MS, equivalent isotopic counts are present, no geometric counting errors occur, and optimal reference sample withdrawal rate is obtained. (Estimated preliminary cats: 5)

Specific CBF Experiments

1. The effect of missile wound upon regional CBF (rCBF): (Estimated cats: 32)
   a. After suitable anesthesia, tranquilization, preparation and placement in a stereotaxic frame, both control and experimental cats will have MS injections for control rCBF measurements. In controls, groups of cats will be used to establish subsequent rCBFs at 10 min, 30 min, 1 hr, 2 hr, 3 hr, 6 hr after placement in the stereotaxic frame. After a control CBF, experimental cats will receive a missile wound to the brain and groups of cats will be used to establish rCBF at the following postwounding times: 10 sec, 10 min, 30 min, 60 min, 2 hr, 3 hr, 6 hr.
2. The effect of dexamethasone, mannitol and DMSO on rCBF following a missile wound (Estimated cats: 42)
   a. Controls will be suitably prepared. Shortly after being in the stereotaxic frame, a MS bolus will be injected to obtain control rCBFs. 1 hr later we will give either dexamethasone 5 mg/kg, mannitol 0.5 gm/kg 25% solution, or DMSO 0.5 gm/kg in 50% solution. We will again measure rCBF 30 and 60 min later
   b. Experimental cats will be prepared and placed in the stereotaxic frame. After a control rCBF they will receive a brain missile wound. One hour later they will be given either dexamethasone, mannitol or DMSO. rCBFs will be measured 30 min and 60 min after this drug therapy.
3. CBF Autoregulation Following a Missile Wound to the Brain (Estimated cats: 26)
   The potential importance of CBF autoregulation to the combat soldier has been stated, (p. 5). In the following experiments we will subject cats with a missile

wound to the brain to moderate (MBP 80 mm Hg) and severe (MBP 60 mm Hg) hemorrhagic hypotension and document the cat's ability to maintain CBF in different parts of the brain. We will see whether dexamethasone, mannitol or DMSO improve impaired CBF autoregulation.

a. Control nontreated cats will be prepared and placed in a stereotaxic frame. MS will be injected for control CBFs. Following this we will bleed the cats to 80 or 60 mm Hg over 30 min. The shed blood will be kept warm and heparinized. The requisite MBP will be maintained 30 min and a 2nd CBF will be measured. Following this the blood will be returned and when normal MBP has been achieved a final CBF will be measured 20 min later

b. Experimental Nontreated Cats will be prepared and placed in a stereotaxic frame. Control CBF will be obtained and the cats will sustain a brain wound. Immediately thereafter the cat will be bled to 80 or 60 mm Hg over 30 min. After either of these MBPs has been maintained 30 min a 2nd CBF will be measured. Shed blood will be returned and a final CBF measured 20 min later.

4. CBF Autoregulation Following a Missile Wound; Treatment with Dexamethasone, Mannitol or DMSO (Estimated cats: 70)

a. Control Treated Cats: After suitable preparation we will measure a control CBF. The animals will be bled to MBP of 80 or 60 mm Hg. After 1 hr at this MBP a 2nd CBF will be measured. The cat will then be given i.v. dexamethasone 5 mg/kg, mannitol 0.5 g/kg bolus in 25% solution, or DMSO 0.5 g/kg bolus in a 50% solution and a final CBF determined 20 min later.

b. Experimental Treated Cats: After preparation we will measure a control CBF. The cats will receive a missile wound and will then be bled to a MBP of 80 or 60 mm Hg. After 1 hr at this MBP a 2nd CBF will be measured. The cat will then be given dexamethasone 5 mg/kg, mannitol, 0.5 gm/kg in 25% solution or DMSO 0.5 g/kg in 50% solution.

03 YEAR: Qualifications of BBB Disruption by a Test Missile

The BBB provides one of the brain's chief homeostatic mechanisms[65] and is thought to be formed from cerebral capillary endothelial cells including their so-called "tight junctions."[66] Lipid soluble substances pass the BBB with ease[67] but nonlipid soluble molecules cross much less readily by diffusive flow and mediated transport.[68] Vesicular transport across the BBB is normally unimportant.[68]

If a substance dissolved in plasma perfuses a capillary bed and if a portion of that substance is removed from the plasma while transiting the capillary bed the substance is "extracted."[69] Molecular extraction is proportional to capillary surface area S and capillary permeability, P. Extraction is inversely proportional to blood flow, F:

$$PS = -FVc\ln(1-E) \qquad (5)$$

Vc = fractional fluid volume in which solute is dissolved; if plasma Vc = 1-Hematocrit.

Thus, the passage of substances across the BBB may be quantified by various PS products and BBB permeability may be described in terms of PS products for a number of test molecules.

We will define the cat's BBB permeability by PS products of the following [3H] test molecules : aminoisobutyric acid, AIBA, (MW 103.1); polyethylene glycol, PEG, (MW 400) and PEG (MW 900). These molecules are not metabolized by the cat[70] and back diffusion (brain-blood) is minimal. We will determine the blood tissue transfer constants (Ki) of these molecules normally and after a missile wound to the brain by the Ohno technique[45] wherein an i.v. bolus of test molecule is given and the subsequent arterial concentration (Ca) is measured over time. At experiment's end, brain concentration of the isotopic molecule (Cb) is determined. With negligible back diffusion:

$$Ki = \frac{Cb}{\int_0^t Ca\,dt} \qquad (6) \qquad\qquad E^{71} = \frac{Ki}{FVc} \qquad (7)$$

In order to obtain a true Cb, retention of test molecule within the brain vascular space (BVS) must be determined and subtracted. BVS may be quantified by [113m] Indium (In). transferritin, MW 58,000.[70,72]

$$\text{BVS} = \frac{[113\text{m In}]\ /\text{mg brain}}{[113\text{m In}]\ /\text{ml plasma}} = \frac{\text{ml plasma}}{\text{mg brain}} \qquad (8)$$

$$\frac{[3\text{H}]\ \text{Test Molecule (TM)}}{\text{ml plasma}} \times \frac{\text{ml plasma}}{\text{mg brain}} = \frac{[3\text{H}]\ \text{TM}}{\text{mg brain}} \begin{pmatrix}\text{owing} \\ \text{to BVS}\end{pmatrix} \qquad (9)$$

True TM Cb:

$$\frac{[3\text{H}]\ \text{TM}}{\text{mg brain tissue}} - \frac{[3\text{H}]\ \text{TM}}{\text{mg brain}} \begin{pmatrix}\text{owing to} \\ \text{BVS}\end{pmatrix} = \frac{[3\text{H}]\ \text{TM}}{\text{mg brain parenchyma}} \qquad (10)$$

Because AIBA, PEG 400 and PEG 900 are essentially diffusion-limited molecules, their PS products will be but slightly affected by CBF.[73,74] We will, nevertheless, measure CBF concomitantly because a missile wound may cause marked CBF changes in certain areas. We will also be able to determine E directly.[71] We will use [14C] iodoantipyrine (IAP)[75] to measure CBF:

$$\text{CBF} = \frac{[14\text{C}]\ \text{IAP brain}}{\int_0^t [14\text{C}]\ \text{IAP blood}\ dt} = \frac{[14\text{C}]\ \text{brain}}{\text{mg brain}} \times \frac{\text{syringe withdrawal rate}}{[14\text{C}]\ \text{blood}} \qquad (11)$$

[14C] IAP blood will be determined by a constant withdrawal speed reference syringe.

Conditions Affecting the BBB: Ischemia,[76] cold lesions,[77] surgery,[78] osmotic agents,[79] hypercapnea,[80] hypertension,[81] and convulsions[82] increase BBB permeability. I can find find no experiments which quantitate the effect of a missile wound upon the BBB despite the high mortality associated with these wounds and the importance of the BBB to brain function. Clearly the BBB will be disrupted about the missile track but the extent of this disruption and the possible occurrence of increased BBB permeability in crucial (brainstem) areas at a distance has not been examined.

The Experiment; Isotopes: We will obtain [14C] IAP,[X] dissolve aliquots (ethyl acteate/benzene) and run the isotope through a Biosil A[Y] (200-400 mesh) column. Purity of the selected fractions will be checked with paper chromatography.[Z,83] Purified isotope will be dissolved in Hepes buffered saline (pH 7.5) such that 0.5 ml contains 25 μCi [14 C] IAP. These will be frozen until used. [113 m In][X] will be obtained by 0.04 N HCl elution from a [113 Sn] generator. 25 μCi of the eluent will be buffered to pH 7.40 (Hepes) and mixed with 0.2 ml freshly prepared cat plasma. The [113 m In] combines with the cat plasma protein (transferritin). [3H] AIBA[X] purity will be checked by ascending paper chromatography with appropriate solvents. Migration of radiolabelled AIBA will be checked against unlabelled AIB migration.[84] We will use samples > 98% purity. For 3H PEG(MW 400; MW 900),[X] the radiopurity of these isotopes will be assayed by gel filtration on Sephadex G-50 and subsequent paper chromatography. All [3H] test molecules will be given as 125μ Ci doses. 3HPEGMW 400 will be specially synthesized by New England Nuclear.

In a separate set of experiments we will inject isotopes i.v. into cats to make sure that the [3H] remains attached to the test molecule when circulating in the cat. All isotopes will be stored at 4°C. [3H] doses will be 5X[14 C]. All reagents will be Aldrich[BB] gold label or equivalents.

Cat Preparation: Cats will be anesthetized with halothane/oxygen. Bilateral femoral artery and vein cannulas (PE90) will be inserted and a brachial catheter (PE50) placed for BP. We will insert a tracheostomy and place a right epidural balloon for ICP measurements.[19] Nupercaine will be applied to all wounds and the extremities casted to protect the catheters. Control and experimental cats to be placed in the stereotaxic frame will then be given phencyclidine 1-2 mg/kg, the halothane will be stopped and 2 hours allowed for halothane excretion. These cats will then be given methahexatol 30 mg/kg i.v., attached to a cat respirator (N_2O/O_2::70/30) placed through an opened decapitator and secured to a stereotaxic frame. The saline filled epidural balloon for ICP will be connected to a transducer placed at earbar level; the right femoral catheter will be attached to a saline-filled syringe in a constant-speed blood-withdrawal pump. 25 minutes before [3H] test molecule administration the left femoral catheters will be converted to an A-V shunt by connecting them to a silastic tubing through which blood can be intermittently sampled. Once the shunt is functional we will heparinize the cat. 20 minutes prior to the end of each experiment [3H] test molecule will be given and blood intermittently sampled from the A-V shunt to give $\int_0^T$[3H] Cadt. 90 seconds prior to the experiment's end, 25μCi [113 mIn] . transferritin will be given i.v. to give

[113 mIn] /ml plasma via a terminal blood sample. 10 seconds prior to the end of the experiment [14C] IAP will be given by a constant i.v. injection; arterial blood will be sampled from the femoral artery cannula attached to the syringe in the constant withdrawal pump. This will yield $\int_0^t$ [14C] Cadt. The cat will be killed by decapitation at experiment's end. Terminal Hct and ABGs will be obtained. We will quickly remove the brain, harden it in dry-iced Freon 12, sample it as per Table 1 and place the brain samples in tared vials which will be immediately reweighed. Plasma samples will be spun and plasma aliquots also placed in counting vials. Brain and blood samples for [113 mIn] transferritin will be counted in the gamma counter. [113 mIn] counts after elapsed time (C_E) will be converted to counts at 0 time, C_0, by $C_E = C_0 \exp(-0.693t/T)$. T = 113 mIn half life; t = elapsed time. After [113 mIn] decay, blood and tissue samples will be dissolved in 0.5 ml Protosol.[X] 0.5 ml of 0.5N HCl will be added to achieve pH of 6.0; Aquasol II[X] scintillant will be added and the samples will be counted with a Beckman[W] Scintillation counter after being placed in the dark for 8-12 hrs. Counts/minute (cpm) will be converted to disintegrations/minute (dpm) by compensating for quench with an external standard. Calculations of Ki, CBF, E and PS will be done on an Apple II computer.

Control Cats: Regional brain PS products for each [3H] molecule will be determined in 5 awake cats (15 cats), 5 cats each after anesthesia and phencyclidine (15 cats) and 20 min, 60 min and 6 hr after being prepared and placed in a stereotaxic frame under N_2O/O_2 analgesia (45 cats)

Experimental Cats: PS products for the 3 test molecules will be measured 20 min, 60 min, and 6 hr following a missile wound. The 20 min PS determination may allow elucidation of transient BBB opening consequent to the missile wound (45 cats).

The [3H] test molecule will be delivered as a pulse 20 min prior to sacrifice. The best sacrifice time, 20 min, 15 min, 25 min, etc., will have to be empirically determined for both control and missile wounded cats. This will require an additional 60 cats (20 for each [3H] test molecule).

In preliminary experiments we will compare CBF as determined by microspheres and [14C] IAP. If[14C]IAP cannot be used to measure CBF because of widespread BBB breakdown in missile-wounded cats we will measure CBF in these animals by microspheres, let the radioactivity of the microspheres decay (10 half lives) and then count for 14C and 3H by scintillation counting.

Data Analysis - All electrolyte, CBF and BBB PS product data will be analyzed by Student's test for paired data. I have worked extensively with Mr. William Johnson of the Department of Biometry at LSU and will continue to work with him on this project.

Facilities - All experiments will be done in 2 laboratories at LSU Medical School, New Orleans. One lab will serve primarily as a shooting range. It will be appropriately armored. The other will house necessary equipment; balances, radioactive counters, blood gas machine, requisite supplies plus desks for the postdoctoral research associate and laboratory technician.

MILITARY SIGNIFICANCE

Several aspects of combat medicine may be manipulated to decrease mortality of the wounded:

1. Rapid evacuation - despite the ubiquitous use of helicopters in Vietnam with speedy evacuation (90% < 4 hr), Vietnam neurosurgical mortality showed no improvement over much of the 1944-1945 ETO experience.

2. Improved hospital facilities/supplies - in Vietnam these were optimal; nevertheless, CONUS-type hospital and operating room equipment, unlimited blood and surgical supplies plus sophisticated anesthetics and unlimited antibiotics failed to result in lowered neurosurgical mortality.

Because evacuation and facilities-equipment-supplies were optimal in Vietnam, it is unlikely that further development of these factors will lead to reduced neurosurgical mortality in the future. Furthermore, in any future major conflict it is doubtful whether evacuation techniques and deployed facilities will be as (literally) luxurious as in Vietnam. If sole reliance for the reduction of neurosurgical mortality is placed on these factors and they fail because of tactical considerations, brain wound mortality will rise.

3. Improved neurosurgical techniques - in reality the technique of brain debridement has changed little since Cushing's time in WWI, 65 yrs ago. Extremely sophisticated neurosurgical techniques possible in civilian hospitals will not be appropriate in forward neurosurgical units. The drop in neurosurgical mortality from WWI to WWII was because of antibiotics rather than surgical advances *per se*.

4. Better understanding of the pathophysiology of the brain wound - contrasted to the above, enhanced understanding of the brain wound may enable combat physicians to effect better treatment of the brain wound. With the development of a standa; . brain wound model, future medical treatment modes for the brain wound will be based on well founded, physiologic principles rather than empiricism or worse. Knowledge of how specific drugs improve physiologic function of the damaged brain may be especially important for the future because large numbers of brain-wounded may have to wait for long periods of time before obtaining definitive neurosurgical care. Under these circumstances appropriate medical manipulation may limit brain deterioration and consequent mortality. Furthermore, such treatment may result in better neurologic function among survivors allowing them to have a better quality of life.

This project will begin to delineate specific, important pathophysiologic derangements of brain function consequent to a brain missile wound. We will test 3 drugs, (dexamethasone, mannitol, DMSO) which have been used to treat some brain conditions, to see whether they are efficacious in reducing specific physiological brain dysfunctions consequent to a brain missile wound. This project will provide the first steps in providing a comprehensive delineation of the pathophysiology of brain wounding caused by conventional weapons and optimal treatment. Hopefully, knowledge gained will result in a significant reduction of wartime neurosurgical mortality from 10 to perhaps 5% or less.

The cost of the proposed research is considerable but to place it in perspective, one must consider that the U.S. government is spending 3 million dollars on a follow-up study of Vietnam-incurred brain wounds. While such a study may provide interesting neuropsychological data it is unlikely to lead to direct improvement in brain wound care. My project will form the basis for a better understanding and treatment of the combat-incurred brain wound. This project is designed to provide information immediately transferrable to the clinical setting. If, because of this project, $1000.00 ultimately could be saved in the care of each brain-wounded soldier the savings to the Army could be considerable:

Type of War	Savings
3000 brain wounded-Vietnam	3 million
12,000 US-WWII	12 million
50,000 German-WWII	50 million

Furthermore if specific drugs are found to be efficacious in the treatment of brain-wounds the Army could concentrate on buying, storing, and supplying these rather than nonefficacious drugs. This would provide additional monetary savings.

The Future

Knowledge gained in elucidating and treating physiological disruptions in the brain consequent to a conventional missile wound can be applied to a laboratory model for studying the optimal treatment of nonconventional brain damage (microwave, lasers). Future wars may be fought with these weapons. The head will again receive 20% of all "hits." The US Army Medical Corps must know how to optimally treat brain wounds inflicted by these newer weapons.

ADDENDUM

Animal Care and Disposal

Cats obtained from Louisiana by the LSU animal care department will be guaranteed 14 days and will be vaccinated against rabies, rhinotracheitis, and distemper. They will be checked for parasites. Both domestic and commercial cats will be kept one per cage. The animal room will be kept 72-74°F under ambient humidity. The light and dark cycles will be 12 hrs each. Two veterinarians supervise the animal care facilities which are AALAC accredited.

At the termination of each experiment the test cats will be placed in plastic bags. Bags containing radioactive cats will be so marked with the amount of contained radioactivity. The animals will be disposed of in accordance with guidelines set forth by the LSU radioisotope committee (Dr. Paul Hyde).

Radioisotopes

All radioisotopes will be stored and handled in an approved fashion. Laboratories will be monitored for radioactivity contamination regularly. The primary investigator has taken and passed the 3 month LSU radioisotope course.

BRIEF SUMMARY OF EXPERIMENTS

01 Year- Histology of Experimental Missile Wound;
- Establish Regional Brain Sampling areas
- Brain Electrolytes Following a Brain Missile Wound
- Effect of Treatment

	MONTHS	CATS
1) Preliminary Experiments to set up missile launcher; perfect "Standard" Brain Wound	1-2	10
2) Gross and microscopic morphology of the missile wound (establish specific brain areas for tissue sampling in all subsequent experiments)	3-4	25
3) Brain Electrolytes 1) Normal 2) Following a missile wound 3) After wounding and treatment	5-12	125
4) Failed Experiments		20
	TOTAL CATS	180

01 YEAR: BRAIN ELECTROLYTES (eg Cerebral Hemisphere Zone 2)*

SMAPLE DATA TABLE 01 YEAR EXPERIMENTS

	Untreated		Treated					
			DEXAMETHASONE		MANNITOL		DMSO	
	CONTROLS	BRAIN WOUNDED	CONTROL	BRAIN WOUNDED	CONTROL	BRAIN WOUNDED	CONTROL	BRAIN WOUNDED
	% H_2O Na K Cl	% H_2O Na K Cl	% H_2O Na K Cl	% H_2O Na K Cl	% H_2O Na K Cl	% H_2O Na K Cl	% H_2O Na K Cl	% H_2O Na K Cl
CONTROL	— — — —							
10"		— — — —						
10'		— — — —						
30'	— — — —	— — — —						
60'	— — — —	— — — —						
2hrs	— — — —	— — — —						
3hrs	— — — —	— — — —	— — — —	— — — —	— — — —	— — — —	— — — —	— — — —
6hrs	— — — —	— — — —	— — — —	— — — —	— — — —	— — — —	— — — —	— — — —
Cats	30	35	10	10	10	10	10	10
							TOTAL CATS	125

* 14 Brain Regions

BRIEF SUMMARY OF EXPERIMENTS

02 YEAR

	CATS
1) Regional Cerebral Blood Flow (CBF)	
A) Untreated	
1) Controls	16
2) Missile wounded	16
2) Regional CBF	
A) Treated	
1) Dexamethasone	14
2) Mannitol	14
3) DMSO	14
3) Regional CBF Autoregulation following a missile wound	
A) Untreated	
1) Controls	13
2) Missile wound	13
4) Regional CBF Autoregulation	
A) Treated	
1) Dexamethasone	26
2) Mannitol	26
3) DMSO	26
5) Failed Experiments	7
TOTAL CATS	185

02 YEAR :

REGIONAL CEREBRAL BLOOD FLOWS

(Specific Brain Region) *

SAMPLE OF DATA 02 YEAR EXPERIMENTS

Sacrifice time after anesthesia or wounding

	UNTREATED		TREATED						
			Dexamethasone		Mannitol		DMSO		
	Control	Missile Wounded	Control	Missile Wounded	Control	Missile Wounded	Control	Missile Wounded	
Control	—	—	—	—	—	—	—	—	
Anesthesia									
10"	—	—							
10'	—	—							
30'	—	—							
1hr	—	—	—	—	—	—	—	—	
2hrs	—	—	—	—	—	—	—	—	
3hrs	—	—							
6hrs	—	—							
CATS	16	16	7	7	7	7	7	7	= 74

* 14 Brain Regions

02 YEAR : REGIONAL CBF AUTOREGULATION FOLLOWING A MISSILE WOUND

(Specific Brain Region)*

	UNTREATED		TREATED						
			Dexamethasone		Mannitol		DMSO		
	Control	Missile Wound	Control	Missile Wound	Control	Missile Wound	Control	Missile Wound	
Control	___	___	___	___	___	___	___	___	
MBP/80mmHg	___	___	___	___	___	___	___	___	
Re-Infuse	___	___	___	___	___	___	___	___	
Control	___	___	___	___	___	___	___	___	
MBP/60mmHg	___	___	___	___	___	___	___	___	
Re-Infuse	___	___	___	___	___	___	___	___	
CATS	13	13	13	13	13	13	13	13	=104

SAMPLE OF DATA 02 YEAR EXPERIMENTS

*14 Brain Regions

03 YEAR - BLOOD BRAIN BARRIER POROSITY AS DEFINED BY PS PRODUCTS OF 3 TEST MOLECULES

(Specific Brain Region*; eg Mesencephalon)

PS PRODUCTS

DATA TO BE OBTAINED 03 YEAR EXPERIMENTS

	Aminoiso-butyric Acid (MW 103)		Polyethylene Glycol (MW 400)		Polyethylene Glycol (MW 900)		
	CONTROL	BRAIN WOUNDED	CONTROL	BRAIN WOUNDED	CONTROL	BRAIN WOUNDED	
Awake	____		____		____		
Anesthesia	____		____		____		
20'	____	____	____	____	____	____	
1hr	____	____	____	____	____	____	
6hrs	____	____	____	____	____	____	
CATS	25	15	25	15	25	15	(120)

*There will be 14 brain regions

not equivalent. Tissue plasma can be determined from [1251] albümin or [113 mIn] . transferritin and tissue rbc from [51 Cr] rbcs. The details of these methods are set forth in his paper. Several additional cats will be used to estimate tissue plasma and tissue rbc concentrations in all sampled brain areas, denoted in Table 1. This will provide accurate Vc data for each brain area.

5) Neither Crockard[19] nor Djördjevic[18] observed significant intracranial bleeding in their brain missile wounds (dogs, monkeys). Nevertheless, the cats must be heparinized for the 03 year Ohno experiments (to keep the A-V sampling shunt open). Therefore, the shunt will be connected and the animal heparinized only ~ 25 minutes prior to the end of the experiment. This will give a minimum of 10' and a maximum of > 5 hours for the blood to clot in the missile wound track. The amount of bleeding in heparinized, wounded brains will be compared to nonheparinized, wounded brains.

6) A constant humiditity must be maintained to maintain constant brain water during dissection. This will be accomplished by a humidity chamber, the construction of which was outlined by Dr. Pappius. She felt that by this hood 40-50 brain samples could easily be taken from each brain without water loss. (phone conversation, August 1982)

7) Control Cats - Adequate control cats for all experiments greatly increase the number of cats required. Every effort will be made to reduce the numbers of controls required by combining data where possible. Measurements from 6 hours control cats will be made first and if these control values are normal fewer shorter time period cats will be required (assuming that if cats maintain normal brain physiology for 6 hours they will for 3 or 1 hour).

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EXPERIMENT NO. 10 DATE 2/2/82

TYPE N_2O/O_2 WATER EXTRACTION BY THE BRAIN

SPECIMAN		WT F	WT E	WT N	DPM ^3H	DPM ^{14}C	DPM PER Gm ^3H	DPM PER Gm ^{14}C		CBF	Ki	E
medulla R	70	17.7076	17.1834	0.0242						0.7857	0.6535	0.8317
medulla L	71	16.9372	16.9100	0.0272						0.9638	0.7688	0.7977
[redacted]												
pons R	72	17.2113	17.1604	.0509						0.9830	0.8460	0.8606
pons L	73	17.2480	17.1903	0.0577						1.0416	0.9000	0.8641
[redacted] ereb R	74	17.1927	17.1631	0.0296	—					0.6552	0.5389	0.8225
ereb L	75	17.1289	17.0534	0.0755						0.6534	0.5757	0.8806
nt verm	76	17.1494	17.0950	0.0544						0.7435	0.6722	0.9041
ost verm	77	—	17.0604	—						—	—	—
[redacted]										—	—	—
nesen R	78	17.1203	17.0772	0.0431						0.8283	0.7160	0.8644
nesen L	79	17.3350	17.2906	0.0444	3					0.8904	0.8102	0.9099
[redacted]										—	—	—
bas gng R	80	17.0636	17.0304	0.0332			—			0.6168	0.5319	0.8623
bas gng L	81	17.2459	17.2085	0.0374						0.7208	0.6386	0.8860
Thal R	82	17.1631	17.1305	0.0326						0.7792	0.6590	0.8457
Thal L	83	17.2565	17.2291	0.0274						0.6397	0.5870	0.9177
[redacted]										—	—	—
mantle 1 R	84	17.0466	17.0323	0.0143					—	0.4743	0.3955	0.8338
mantle 1 L	85	17.1879	17.1703	0.0176						0.7299	—	—
mantle 2 R	86	17.1307	17.1022	0.0285						0.5668	0.4668	0.8236
mantle 2 L	87	17.1505	17.1091	0.0414						0.9879	0.8683	0.8789
[redacted]										—	—	—
mantle 3 R	98	17.1172	17.0475	0.0697						0.4571	0.4250	0.9724
mantle 3 L	89	17.2098	17.1809	0.0289						1.1768	0.9693	0.8237
mantle 4 R	90	17.1722	17.1299	0.0423						0.4316	0.3964	0.9194
mantle 4 L	91	17.1037	17.0476	0.0561						1.0572	0.9005	0.898
[redacted]									$\bar{x}$	0.7331	0.6317	0.8617
3CK [redacted]									SD	0.3266	0.2656	

Sou ce of Equipment And Materi s

A) Harris-Lake Inc.- Cleveland, Ohio

B) Ayerst Laboratories-New York, New York 10017

C) Clay Adams- (Division of Becton Dickinson Co) Parsippany, N.J. 07054

D) E and M Instrument Co. Inc.- Houston,Texas

E) ibid

F) Ciba Pharmaceutical Co- Summit, N.J. 07901

G) Johnson and Johnson Co- New Brunswick, N.J.

H) Parke Davis, Morris Plains, N.J.
NOTE: if phencyclidine unavailable we will us Ketamine HCI, Bristol Laboratories Syracuse N.Y.

I) David Kopf Instruments, Tijunga, Ca 91042

J) Harvard Apparatus Co. Inc.- Millis, Ma 02054

K) Instrumentation Laboratories, Lexington Ma

L) Astra Pharmaceutical Products, Inc.- Worcester, Ma

M) J.T. Baker Chemical Co.- Phillipsburg, N.J. 08865

N) Eli Lilly And Co.- Indianapolis, In

O) Merk & Company, West Point, PA

P) McGaw Co, Division AH Supply- Irvine Ca

Q) "Spectranalyzed" grade MC/B- Norwood, Ohio

R) Precision Scientific c/o American Scientific Products

S) Harvard Infusion Withdrawl Pump 935

T) Codman & Company - Randolph, Massachusetts 02368

U) Ean M Instruments Inc.- Houston, Tx

V) Minnesota Mining & Manufacturing Co.- St. Paul, Minn.

W) Beckman Instruments Inc.- Irvine Ca 92713

X) New England Nuclear, Boston, Ma 02118

Y) BioRad Laboratories, Richmond Ca

Z) Whatman Ltd, England

AA) Buchler Cotlove- Fort Lee, N.J.

BB) Aldrich Chemicals Co- Milwaukee, Wisconsin 53201

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AWARDS:	Army- Bronze Star (Service) Purple Heart Vietnam Technical Service Medal Army Commendation Medal
GRANTS:	N.I.H. Grant # NS11647-04 with Charles I. Berlin, Follow up studies on selected men who sustained a brain wound in Vietnam, 1977 to present: Bel. Award Louisiana Heart Association 1981-1982 Cerebral blood flow and water extraction.
ORGANIZATIONS:	Congress of Neurological Surgeons American Association of Neurological Surgeons Society of University Neurosurgeons Neurosurgical Society of America Southern Neurosurgical Society Louisiana Neurosurgical Society-President Fellow, American College of Surgeons Louisiana Medical Society American Medical Association Royal Society of Medicine, London Founder Member, International Society of CBF and Metabolism
MASTER'S THESIS (1970):	Brain Abscesses at the University of Minnesota Hospitals, 1946-1965

SABBATICAL (AUGUST 1978 - AUGUST 1979):

King's College, London with Professor Hugh Davson working on:
1) Effect of hypoglycemia upon cerebrospinal fluid production, iodide clearance and brain electrolytes
2) Amino acid clearance from cerebrospinal fluid

HOSPITAL STAFFS:	Charity Hospital, New Orleans, Louisiana Southern Baptist Hospital, New Orleans, Louisiana Hotel Dieu Hospital, New Orleans, Louisiana

EXAMINER, NEUROSURGICAL BOARDS:

September 1980

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PUBLICATIONS

Neurosurgical Infections

1. *Carey, M.E., Chou, S.N., French, L.A.: Long term, neurologic residua in patients surviving brain abscess with surgery. J Neurosurg 34:652-656, 1971*

2. *Carey, M.E., Chou, S.N., French, L.A.: Brain abscesses seen at the University of Minnesota Hospitals 1946-1965. J Neurosurg 36:1-10, 1972*

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8. Carey, M.E.: The treatment of brain abscess, in (ed) Meacham W.- As yet untitled book on brain infections

-6-

PUBLICATIONS

Others

1. *Carey, M.E., Nance, F.C., Kirgis, H.D., Young, H.F., Megison, L., Kline, D.G.: Pancreatitis following spinal cord injury. J. Neurosurg 47:917-922, 1977*

2. *LaCour, F., Trevor, R., Carey, M.E.: Arachnoid cyst and associated subdural hematoma. Arch Neurol 35:84-89, 1978*

3. *Schecter, F.G., Carey, M.E., Bryant, L.R.: Bilateral apical intrathoracic masses associated with Von Recklinghausen's disease. Chest 75:367-368, 1979*

4. *Carey, M.E.: Brain Trauma in Practice of Medicine, Hagerstown, Md. Harper and Row, Publishers, Inc., 1979*

5. *Correa, A.J.E., Rodriguez, M., Carey, M.E.: SIADH after subarachnoid hemorrhage and craniotomy. South Med J 73:932-934, 1980*

6. Carey, M.E.: Brain Trauma in Spittell, J.A. Jr. (ed) Clinical Medicine Philadelphia, Harper and Row, Publ., 1981 (Chapter 26)

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Abstracts

1. Carey, M.E., Vela, A.R.: The effect of multiple levels of arterial hypotension upon the rate of cerebrospinal fluid formation in dog. Fed Proc 33: 360, 1974

2. Vela, A.R., Corales, R.L., Carey, M.D.: The effect of cerebral venous drainage obstruction upon cerebrospinal fluid accumulation. Fed Proc 34:397, 1975

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5. Vela, A.R., Carey, M.E., Walker, K.: The effect of hypotension upon ventricular absorption of phenosulfonphthalein. Fed Proc 36:570, 1977

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Talks Presented
(Continued)

16. *Vertebral Osteomyelitis. American Association of Neurological Surgeons, Miami, April, 1975.*

17. *Prophylactic Antibiotics in Neurosurgery. American Association of Neurological Surgeons, New Orleans, Louisiana, April, 1978.*

18. *Treatment of Brain Abscess (Seminar). American Association of Neurological Surgeons, Los Angeles, California, April, 1979.*

19. *Comments on the Production of Experimental Brain Abscess. American Association of Neurological Surgeons, New York, N.Y. April 1980.*

20. *Effect of Severe Hypoglycemia on CSF Formation, Ventricular Iodide Clearance and Brain Electrolytes. Erwin Riesch Symposium, Berlin, July, 1980.*

21. *War Medicine and Combat Neurosurgery. Invitation of Surgeon General, US Air Force; Wilford Hall Hospital, San Antonio, Texas, March 1981*

22. *Neurosurgery in Vietnam, Uniformed Services Medical School, Bethesda, MD.,* October 1981

23. *Treatment of Brain Abscess. American Association of Neurological Surgeons, Boston, Mass., April 1981.*

24. War Medicine and Combat Neurosurgery. Invitation of Surgeon General, US Air Force, Munich, Germany August 1981

25. An Analysis of Fatal and Non-fatal Head wounds Incurred during Combat in Vietnam by US Forces. 4th International Ballistics Symposium Gothenberg, Sweden September 1981

26. War Medicine and Combat Neurosurgery. Invitation of Surgeon General, US Air Force Andrews AFB , MD, October 1981

Visiting Professor

University of Kentucky, April, 1975

TABLE OF CONTENTS

:ILITARY BACKGROUND

In Army combat, head wounds are the most lethal and cause almost half of all immediate deaths,[1-4] ig. 1. The head accounts for only 9-12% of the exposed body area in combat[3] yet receives 20% of all its',[1,2,4,5,6], Fig. 1. In civilian [7] or rear echelon Army hospitals [8], patients with closed head injuries are ore common than open wounds. In combat, however, especially in forward Army hospitals, penetrating rain missile wounds are far more common. During WWII in forward hospitals in N.W. Europe, brain missile ounds accounted for 43% of all neurosurgical admissions.[9]

Despite these facts, few studies on the physiological effects of a missile wound to the brain have een done to lessen the mortality and morbidity. Perhaps some people are fatalistic about brain missile ounds, thinking that soldiers with these lesions are "lost," not only to life but to the Army in particular. e agree that a direct bullet wound to the brain is generally fatal because of the bullet's high velocity and nergy of deposit. In major combat, however, more than 70% of all wounds are caused by lower energy nell fragments.[10] Not only do many soldiers who sustain a fragment wound to the brain live, but in WII[11] and Vietnam[12] almost one third of men who received a brain wound from a missile were able to ontinue some form of Army duty after appropriate neurosurgical care. This project focuses on the treat- nent and physiological understanding of a non-fatal brain missile wound in order to further reduce morta- ty and morbidity.

The treatment of war wounds is primarily surgical, but restoration of physiologic function with adjunc- ve medical therapy has proved extremely important for all major combat wounds[13-15]. The modern urgical technique of brain wound debridement was developed in WWI by progressive surgeons[16,17] who dvocated primary debridement with irrigation-cleansing of the brain missile track, removal of necrotic rain and foreign material, and closure of dura and scalp. However, the striking reduction in neurosurgical nortality from 50% in WWI[18-20] to 11-14% late in WWII [9,21] is best explained by the advent of antibiotics adjunctive medical therapy) rather than by any basic improvement in neurosurgical techniques. Neuro- urgical mortality following brain debridement for US forces in Korea was 10%[22] and in Vietnam it was 10 o 12%[23,24]. These later data indicate that no significant reduction in neurosurgical mortality from brain ppears to occur only about the hemispheral missile track. With increasing missile KE, distant damage ccurs in the contralateral hemisphere or brainstem, (Fig. 5). Distant brain damage from missile wounds as been described clinically from Vietnam[26]. The gross and microscopic features of our experimental rain wounds strikingly resemble human missile wounds, (figs. 6-9).

2. <u>We have determined that respiratory arrest frequently occurs with a missile of sufficient energy</u>. ecause we wish to primarily study the physiologic effects of non-fatal brain wounds we have studied the issile's effect on anesthetized, non-paralyzed cats capable of normal respirations. Table 1 indicates that e most important immediate effect of a missile is upon the brainstem and may cause respiratory arrest.

Table 1

Effect of Energy Deposit Upon Respirations

	Energy Deposit (J)	
	0.93	1.35
No Apnea	6	6
Transient Apnea	1	5*
TOTAL	7	11

* fatal without respiratory support
this energy represents the LD_{50}J energy for this wound

t 0.93 J, missiles penetrated the brain but caused mainly local cerebral hemisphere damage; brain-tem effects were minimal and included transient blood pressure changes and bradycardia. At 1.35J, issiles caused increasing right hemisphere damage as well as brainstem dysfunction marked by apnea 45% of cats. Following 60 to 80 minutes of respiratory support <u>all</u> temporarily apneic cats were able resume their own respirations. **This finding may indicate that there is a group of brain-wounded dividuals whose lives may be saved if immediate short-term respiratory support could be provided by e buddy system or by corpsmen.** This phenomenon of apparently reversible apnea following a missile ound deserves detailed investigation because immediate treatment may be lifesaving.

3. Though the literature contains many[27-29] observations on brain swelling after a missile ound, we have not observed significant right cerebral edema up to 24 hours following wounding at .93J or 1.35J. These findings are still preliminary, however, and need to be confirmed with further xperimentation. Concomitant serum osmolality changes after wounding must next be investigated.

feature in their animals. Other brain injury models do not have the spectrum of focal injury plus distant brain damage and systemic effects seen with the missile wound. Cold-induced[53], ischemic[54] or stabbing[55] brain lesions involve the cerebral hemispheres alone; they will not be associated with the brainstem effects which we have shown a missile causes, particularly the all important apnea. Percussion- type injuries[33,56,57] will cause brainstem effects but will not have the concomitant focal cerebral hemisphere damage that makes the missile wound "strikingly different from closed head injury." Conceivably a critical interaction may exist between focal brain damage and brainstem function.

Future improvements in the treatment of brain wounds certainly will be in the realm of adjunctive medical therapy. Our missile wound model, faithfully replicating a fragment in the human, will enable us to: (1) screen drugs that may improve treatment of the brain-wounded; (2) delineate the pathophysiologic effects of a missile wound to the brain so that treatment can be precisely focused on the critically deranged physiologic functions. Low-energy wounds can be used to study physiological and biochemical disruptions associated with the hemispheral wound and their treatment. With higher energy wounds, we can study potentially fatal brainstem events (particularly apnea) to design therapy to prevent them.

[redacted]

Fig.3:(right) Lateral skullfilm of one of our experimental cats. 30mg sphere entered through right frontal bone.

Fig.4: CT scan of experimental cat showing indriven bone in right frontal area. Scan done 4 days following wounding in a surviving cat.

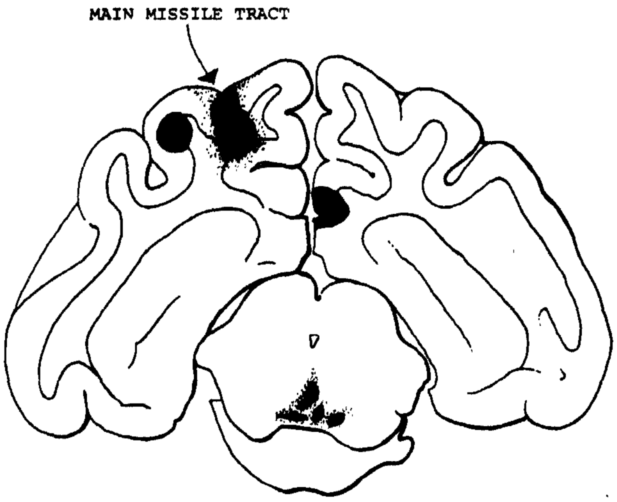
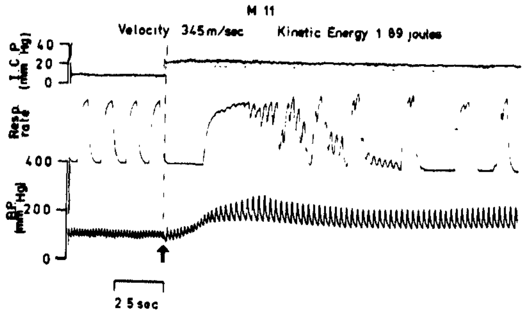


Fig.5: Schematic illustration of areas of hemorrhage (filled areas) and extravasation of Evan's blue (stippled areas), both focally around the main tract and distally in cortical and brain stem regions following brain missile wounding.

Fig.10:(right) Following an experimental missile wound, changes in BP, P, and respirations occur. In this cat, ICP remained elevated.



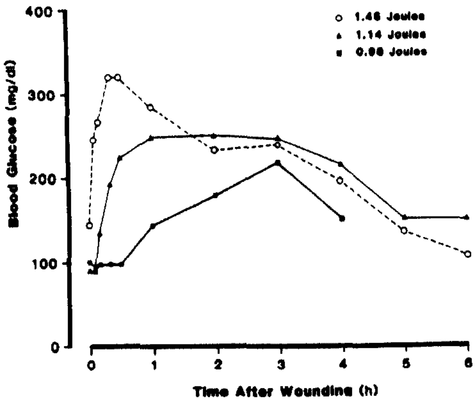
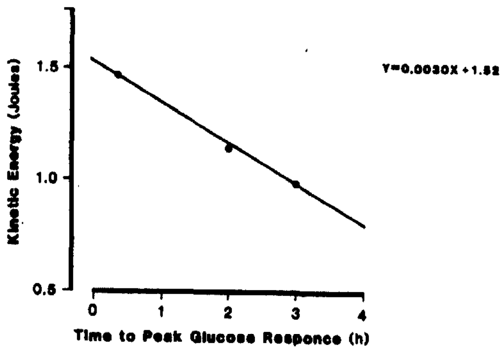


Fig.11:(left) The intensity of post-wounding hyperglycemia appears to be related to wounding energy.

Fig.12:(right) The rapidity of onset of hyperglycemia likewise appears to be related to wounding energy.



OBJECTIVES OF INVESTIGATION

The first objective of this investigation is to detail the neurological status of the animal before and after brain missile wound in order to assess which treatments result in decreased mortality and morbidity. Our second objective is to undertake detailed pathophysiological studies initially in untreated animals and later with treatments shown to be efficacious in reducing mortality and morbidity. By undertaking both neurological and pathophysiological studies concurrently, we will be the first laboratory to attempt a detailed assessment of the pathophysiological basis of the neurological deficits observed following brain missile wounding. This should lead to sound physiologic and pharmacologic methods to ameliorate the bra: damage.

Our studies so far have focused on brain missile injuries in normotensive animals. In this proposal, w will include studies of brain missile wounding in animals subject to post wounding hypotension mean arteri blood pressure (MABP reduced to 40 mmHg for 1 hour) since hemorrhage is an extremely important cause combat death[58]. Two thirds of Dr. Carey's patients in Vietnam had body as well as brain wounds and man were in hypovolemic shock.

The overall concept of our investigation is shown below. Our general methodology of brain wounding will be given first, followed by a discussion of each proposed experiment, including its objectives, methodc logy and data analysis.

GENERAL METHODOLOGY OF ANIMAL PREPARATION AND WOUNDING

Animals Cats weighing between 3-4 kg will be used. They will be housed singly for approximately 14 days prior to experimentation and allowed food and water ad lib. They will be kept on a 12h light-dark cycle (lights on 06:00h). The rationale for using cats as opposed to other animals is given in the section - 'Animal Use Documentation'.

For experiments we will induce anesthesia with 1% Brevital IV. Maintenance will be by intermittent Brevital injection. An endotracheal tube will be inserted following endotracheal injection of 0.5 ml 5% Lidocaine. We will cannulate a femoral artery (PE 90 tubing) for BP recordings (precalibrated Narco RP 1500 and Narco physiograph) and blood sampling, and a femoral vein for saline and drug administration. Colonic temperature will be kept at 37° ± 1°C by a heating blanket. In experimental protocols requiring the animal to be artificially ventilated throughout the duration of the experiment, a muscle relaxant, Pavulon (0.5 mg/kg), will be administered, the cat connected to a respirator-anesthesia machine and anesthesia maintained with $N_20/0_2$ (70/30). Arterial blood gas values (measured by IL micro 313/326) will be maintained within the normal range through the use of sodium bicarbonate (I.V.), respirator adjustments or both. ECG will be monitored (Narco physiograph) and end-tidal CO_2, and respiratory rate recorded (IL 200).

Following anesthesia and specified monitoring, we will place the animal prone in a stereotaxic frame (DKI), anesthetize the scalp with subcutaneous 1% xylocaine, and make a 4 cm right frontal incision for removal of a portion of the anterior right frontal sinus wall.

Brain missile wound A transcranial, right fronto-occipital brain injury will be made with a 1.98 mm steel ball (31.7 mg) fired from a custom-made helium powered gun at a range of 80 cm. The missile will perforate the right frontal bone (0.7 - 0.9 mm thickness) and enter the brain through cortex area A6 (ap + 28 mm, v + 22, L 5 mm: coordinates to Reinoso-Suarez).[123] Missile energy of deposit will be calculated from $\frac{1}{2}mv^2$ where m = mass of missile, and v = velocity (measured with velocity gate).

As indicated above, we will perform all physiological studies on two groups of cats. Some will remain normotensive throughout. Others, the hypotensive experimental group, will be bled at the rate of 2ml/min immediately following wounding to simulate a second, concomitant, hemorrhagic-shock producing wound. We will withdraw blood until the cats MABP reaches 40 mmHg and maintain this MABP for 1 hr. Shed,

2) Neurologic function - Animals surviving their wound will undergo the following neurologic tests. Observers will be unaware of each animal's treatment and parameters listed below will be assessed daily for up to 3 weeks post brain missile wounding. Procedures have been described previously[73] but with modifications.

(A) **Neurological Response**

Motor function	
Cat walks with normal gait-no neurological deficit	6
Cat walks with abnormal gait, has mild hemiparesis	5
Cat barely walks with moderate hemiparesis	4
Cat unable to walk with moderate hemiparesis	3
Cat unable to walk with severe hemiparesis	2
Cat unable to walk with hemiplegia	1
Sensory function	
Cat responds appropriately to tactile and noxious stimuli	5
Cat responds appropriately to noxious limb stimuli only	4
Inappropriate response to noxious limb stimulation	3
Reflex response to noxious limb stimulation only	2
No response to noxious limb stimulation	1
Level of consciousness	
Awake and alert	5
Awake and alert with lack of spontaneous movements	4
Drowsy, responds only to noxious stimuli	3
Stuporous, minimal response to noxious stimuli	2
Comatose	1
Pupillary response	
Unilaterally reactive to light	2
Unilaterally unreactive to light	1

(B) **Activity:** Each cat will be placed in an observation area (6ft x 6 ft) with the floor divided into 9 squares. The number of squares entered (entry by all four paws) will be counted during a 30 minute period for two consecutive days prior to wounding and each day following wounding.

$$\text{Maximum score} = 5 \text{ when the ratio of: } \frac{\text{Number of squares entered post wound}}{\text{Number of squares entered prior to wound}} = 1$$

(C) **Food And Water Intake** Lactated Ringer's solution will be injected subcutaneously to provide adequate daily fluid maintenance until cats begin to eat and drink voluntarily.

(a) Water intake; ½ point for each 4cc per day of water intake (or part thereof) up to a maximum o 5 points

(b) Food intake; ½ point for each 4g per day of food (or part thereof) up to a maximum of 5 points

(D) **Composite Score:** This will be the sum of A, B, C above.

3) Sample data table anticipated for each injury energy is shown:

Separate scores for Apneic or Non-Apneic cats	Time after brain missile wound (days) 1	3	5	7	14	21
Mortality rate						
Motor function						
Sensory function						
Level of consciousness						
Pupillary response						
Activity						
Food and water intake						
Composite Score						

Differences in neurological scores among experimental groups of cats wounded at different energies will be compared using Kruskal Wallis analysis of variance (AOV) followed by The Mann Whitney U-Test.

The values to be obtained for the above table for normotensive cats at 1.35 J will serve as control data for comparisons of the effect of different treatment regimes.

ACUTF PHYSIOLOGICAL STUDIES

Rationale: Physiologic events occurring immediately after the brain missile wound should be fully understood. Central nervous system damage may cause profound systemic derangements e.g. diminished cardia output[49] and hypertension. Whether a brain-wounded person lives or dies may be decided by physiological changes (e.g. hypertension or apnea) occurring within a few seconds, minutes or hours after wounding. Ou experiments have shown that severe respiratory abnormalities may occur with missiles of sufficient energ We will ascertain whether this can be correlated with concomitant hypertension or plasma catecholamine response. Hypertension might increase brain damage and turn a nonfatal wound into a fatal one. Physiologic changes following wounding may indicate brain wound severity and correlations between different pathophysiologic events and mortality, morbidity indices may lead to better treatment. Acute physiological studies will be conducted concurrently with specific treatment evaluations (See page 27). Once efficacious treatments have been identified, their acute physiologic effects will be investigated.

Methods Cats will be prepared as in the general preparation. Additionally, we will insert an epidural pressure transducer (MMI ICT/b), in the occipital midline for ICP. We will place bilateral anterior and posterior stainless steel skull screws (dural contact) for EEG recorded on a Narco or Grass 78 physiograph

We will measure the following up to 6 hrs. after wounding: ICP, EEG, BP, heart rate, and ECG; PaO_2, $PaCO/_2$, pH, HCT, osmolarity, electrolytes, glucose, catecholamines and amino acids. These values will be assessed in 72 normotensive cats as indicated below (18 at each energy level).

	Wound Energy (J)			
	0.7	0.93	1.35	2.8
Non-Apneic (resp. support not needed)				
Apneic (resp. support required)				
Ventilated throughout (paralysis and respirator)				

This combination of experiments will help differentiate respiratory from cardiovascular factors in th observed physiological changes. Having determined the acute physiological effects of brain wounding in normotensive cats, we will study them in hypotensive ones wounded at the hypotensive $LD_{50}J$ (18 cats). Once effective treatments have been identified, their effects on acute physiological functions will be determined in normo and hypotensive cats wounded at respective $LD_{50}J$ energies. Six sham-operated controls will be evaluated to see whether sampling procedures affect variables to be estimated.

Sampling Times will be as follows: Pressure (blood + ICP), heart rate, ECG, EEG, respiratory rate, end-tidal CO_2 - all continuously; $PaCO_2$, $Pa0_2$, pH, urine -every 30 minutes. Blood hematocrit, osmolarity, electrolytes, glucose, catecholamines, and amino acids will be analyzed from 2 ml samples taken -60, -30, 1, 3, 10, 30, 60, 120, 240, 360 minutes following wounding. 2 ml of lactated Ringer's solution will be infused following each sample.

Data Analysis

(a) Temporal changes in each physiological function will be plotted and assessed for each of the experimental groups.

(b) Correlation matrices will be tabulated as shown below for normotensive animals injured with a range of energies:

Factor		MAPB	$EtCO_2$ (Resp)	HR	HCT	OSM	AD	NOR	GLU	AA	Na/K
Wound Energy	r	+	+	+	+	+	+	+	+	+	+
	p										
MABP	r	-	+	+	+	+	+	+	+	+	+
	p										
$EtCO_2$ (Resp)	r	-	-	+	+	+	+	+	+	+	+
	p										
HR	r	-	-	-	+	+	+	+	+	+	+
	p										
HCT	r	-	-	-	-	+	+	+	+	+	+
	p										
OSM	r	-	-	-	-	-	+	+	+	+	+
	p										
AD	r	-	-	-	-	-	-	+	+	+	+
	p										
NOR	r	-	-	-	-	-	-	-	+	+	+
	p										
GLU	r	-	-	-	-	-	-	-	-	+	+
	p										
AA	r	-	-	-	-	-	-	-	-	-	+
	p										
Na/K	r	-	-	-	-	-	-	-	-	-	-
	p										

All coefficients will be calculated after conversion of data to natural logarithm
r = correlation coefficient; p = probability; + values to be obtained; - no value

Tables to be obtained at 1, 3, 10, 30, 60, 120, 240, and 360 minutes following brain missile wounding.

(c) Comparisons of the effect on physiological parameters following missile injury at the LD_{50} energy in hypotensive and normotensive (untreated and treated) animals will be made by AOV. Differences between groups will be determined by Newman-Keul's test.

EFFECTS OF VARIOUS TREATMENTS ON MORTALITY AND MORBIDITY

Rationale A number of drugs improve the functional neurologic recovery following brain concussion[59,60,61], freeze lesions[62,63], spinal cord injury[64-70] and in animal models of cerebral ischemia[71,72,73]. However, with exception of the use of mannitol and dimethylsulfoxide[74], no systematic studies have shown the possible beneficial therapeutic effects of these treatments following a brain missil injury. An acute need currently exists to screen drugs which might improve the function of damaged but not dead brain caused by missile injury. We propose to test the efficacy of different treatments on mortality and morbidity scores following missile wounding.

EFFECT OF VARIOUS TREATMENTS ON MORTALITY AND MORBIDITY IN NORMOTENSIVE ANIMALS

INJURED AT 1.35 JOULES

TREATMENT	MODE OF ACTION	PROTOCOL mg/kg bolus	-- mg/kg/hr
(A) Drugs administered * 1 hour post missile wound			
Naloxone		2	2
TRH	Opiate	2	2
(methyl)-TRH	antagonists?	0.5	0.5
Dynorphin (1-13)		2	0.1
L-dopa	Dopamine precursor	200	200
Dexamethasone	Increased CBF, CO	0.5	0.5
Dimethylsulfoxide	Decreased ICP, blood viscosity	500	500
Mannitol		500	500
Tromethamine	Alkalizing agent	100	25
(B) Drugs administered *24 hour prior to missile wound			
Parachlophenyalanine	Serotonin synthesis inhibitor	150 mg/kg i.p.	
(C) Drugs administered * 1 hour prior to missile wound			
Naloxone	Opiate antagonist	2	2
Phenoxybenzamine	α-adrenergic block	2	2
Atropine	Cholinergic block	0.2	0.2

(D) **Respiratory support:** in animals which show apnea post-wounding

Hyperventilation	Decreased pCO_2	End-tidal CO_2: 3-4%
Hypoventilation	Increased pCO_2	End-tidal CO_2: 6-7%

* We will give drugs as a 0.5 ml IV bolus followed by continuous infusion at 0.5 ml/hr over 4 hours unless stated otherwise.

The timing of a particular drug administration following wounding is of paramount importance in determining the eventual clinical outcome. In combat, initial treatment may be delayed. Nevertheless, treaments of proven efficacy can be carried out by corpsmen. Thus we will initially administer drugs 1 hour post missile wounding. Detailed dose-response and time of administration protocols will be developed once the drug(s) are shown to be effective in reducing mortality and morbidity.

Treatment protocols to be utilized in the present study are summarized (page 28). By these protocols, we hope to improve neurologic function in non-apneic brain-injured cats and to isolate the physiologic mechanisms involved in the apneic response. Our reasoning for use of proposed treatments is briefly summarized.

(A) **Drugs to be administered one hour post missile wound**

Opiate The therapeutic effects of naloxone and TRH are believed to be mediated by their ability to antagonize the release of endogenous opiates[69,70]. In a recent study of concussive brain injury to cats, naloxone significantly reduced the hypotension seen after higher grades of injury[59]. However, no observations were reported on the effect of naloxone on mortality and morbidity scores. Studies by Faden and his co-workers have shown that both naloxone and the 'physiological' antagonist TRH dramatically improve the functional neurologic recovery following spinal injury[64-70]. Of particular interest is the ability of TRH to do this when given 24 hours after traumatic injury[68]. In addition to TRH we propose to use an analogue of TRH, (methyl- his)-TRH, a more potent analogue of TRH with a longer biological half-life[75,76]. Dynorphin (1-13) is an endogenous neuropeptide which is widely distributed in the brain and may act as a regulatory neuropeptide, not as a classical opiate agonist or antagonist[73,77]. In cats with focal cerebral ischemia Baskin et al[73] have demonstrated that dynorphin (1-13) infusion (6 hour post) not only increased survival rates but that these cats showed minimal neurological deficits. All untreated cats in this study died within 48 hours. Dynorphin (1-13) did not influence systemic variables or regional cerebral blood flow suggesting that its effects are mediated directly in the CNS. The extent to which most peptides can penetrate the blood-brain barrier when administered peripherally is controversial[78,79,80]. However, disruption of the BBB following brain missile wounds, as shown in our preliminary studies with extravasation (gross extravasation of Evans Blue fig. 5) would favor entry and possible action of dynorphin (1-13) into these traumatized areas of brain. The integrity of the BBB is to be evaluated in more detail autoradiographically in this proposal (see page 33).

Dopaminergic and serotonergic drugs Many studies have implicated brain dysfunction to disorders of central metabolism of dopaminergic and serotonergic neurotransmitters in patients with severe head injuries[81-87] and in animal models of concussion[88,89] and ischemia[90,91]. Patient studies have shown markedly decreased values for homovanillic acid (metabolite of dopamine) and increased levels of 5-hydroxyindoleacetic acid (metabolite of serotonin) in ventricular and lumbar samples of cerebrospinal fluid. However, of primary interest to this study is the finding that treatment of the chronic phase of head injury with L-dopa (dopamine precursor) has proved to be beneficial[82,83,84].

Others: The possible therapeutic effects of dexamethasone, dimethlysulfoxide (DMSO) and mannitol in the management of head trauma have been topics for research studies for many years[92-95]. Of particular interest are those related directly to brain missile wounding[50,74]. These later studies demonstrated the effectiveness of DMSO and mannitol in reducing mortality following penetrating cerebral missile wounding. The mortality rates were 45% in untreated primates, 25% in mannitol-treated and 14% in DMSO-treated. These pioneering studies showed that gunshot wounds to the head could be effectively treated. Nevertheless in these experiments few animals were studied, wounds were effected through trephined holes, mortality was defined as those animals who survived for only 6 hours after wounding and no subsequent behavioral evaluations were made. Whether these animals really would have lived for protracted periods is unknown. Our animal model allows the examination of both acute and chronic mortality rates and the neurological response following treatment with many drugs including, DMSO, mannitol and dexamethasone.

Tromethamine (THAM) infusion after fluid-percussion injury to the brain in cats improves survival and decreases morbidity[57]. The mode of action is uncertain but the authors postulate that tromethamine has an alkalizing effect which reduces CNS metabolic acidosis due primarily to accumulation of lactate in the brain. We propose to investigate the possible development of cerebral metabolic acidosis in our animal model (see page 33).

(B) **Pretreatment 24 hours prior to missile wound**

The involvement of the serotonergic response to head injury has been described above. Porta et al[82] have implicated the involvement of serotonergic structures in the early stages of brain trauma and serotonin in maintaining edema and vasospasm. We propose to pretreat cats with para-chorphenylalanine, (serotonin synthesis inhibitor) in order to deplete brain 5HT and its turnover by approximately 50%[96] and observe the consequent effects on mortality and morbidity. If 5HT involvement can be confirmed either by this drug procedure or by more detailed investigations proposed (page 33), numerous drugs are available to modify 5HT release, reuptake for administration after brain missile injury.

(C) **Drug treatments 1 hour prior to brain missile injury**

We have chosen to incorporate a 1 hour drug pretreatment plan to evaluate the possible mechanisms underlying the respiratory and cardiovascular events which occur immediately after brain missile injury. Apnea, transient but marked MABP rise, sustained hypotension, arrythmia, bradycardia, and hyperglycemia are physiological responses common to both brain missile injury (our experiments)[44,45] and in animal models of concussive head injury[97,98,99]. These events are presumed to be brainstem mediated. We would anticipate that the acute hypertension and hyperglycemia effects are mediated by a massive sympathetic discharge and may be blocked using an α-adrenergic blocker, phenoxybenzamine. Acute MABP changes of the magnitude observed in our animals are known to cause a breakdown of the blood-brain barrier in animals not subjected to head trauma[100]. The distal lesions observed in our animal model may be a consequence of the abrupt rise in MABP and not directly to a 'shock wave' or 'compression effects' of the missile traversing through the brain tissue. Our proposal includes study of the plasma catecholamine response.

Hockwald[101] has shown that the hyperglycemic response to a stab wound to the brain could be attenuated by α-adrenergic blockade. This response needs further study especially as hyperglycemia itself has been shown to be detrimental to animals subject to focal ischemia[102-107].

(D) **Respiratory support**

The apneic response after brain missile injury at 1.35 joules is reversible following a period of ventilatory support (table 1 page 12). We determined the duration of respiratory support needed by the cats' ability to resume their own respirations. In these experiments mechanical ventilation was adjusted so that end-tidal CO_2 values were within the normal range for control cats, 4-5%. It is important to determine whether animals would require shorter periods of ventilatory support if they were maintained at 5-6% (hypoventilation) or 3-4% (hyperventilation) immediately the apneic response is shown to occur as $PaCO_2$ affects CBF, intracranial blood volume and ICP. We are the first to emphasize the occurrence of apnea (transient or permanent) depending upon missile energy deposited in the brain. We plan to investigate whether pretreatment with an α-adrenergic blocker, cholinergic blocker (atropine) or an opiate antagonist (naloxone) can attenuate the apneic response following brain missile injury. Not only may this shed light on underlying brainstem mechanisms but it may point the way to successful resuscitation of brain wounded individuals who may be temporarily apneic.

<u>Methods</u> Cats will be prepared generally as described on page 22. All will be wounded at 1.35J (LD_{50}). Untreated, control cats will be allowed to recover and their daily neurological behavior scored (scheme on page 24) up to 21 days. Treated cats who survive will similarly have their neurologic behavior scored.

<u>Data Analysis</u> Drug efficacy will be determined by differences in daily neurologic scores between control and treated cats. A sample neurologic scoring scheme has been presented (page 24), indicating methods of data analysis. Behavioral results will be analyzed using the Kruskal Wallis AOV followed by the Mann Whitney U-test to locate specific group differences.

CEREBRAL TRAUMA CONSEQUENT TO MISSILE WOUND

Rationale We will examine disorders in 1) brain metabolites; 2) brain neurotransmitters; 3) regional blood flow; and 4) the blood-brain barrier in an attempt to ascertain which of these major systems required for normal brain function is most disrupted by missile damage. These studies, leading to a deeper understanding of how a missile adversely affects the brain, hopefully will allow the development of better treatment.

Ample evidence exists in the literature that brain energy mechanisms must be intact for normal brain function[108]. Pathologic states which affect the brain energy cycle cause severe neurologic dysfuction[109]. Alterations in brain neurotransmitters may cause abnormal behavior in test animals[110]. Whilst the role of neurotransmitters in regulating CBF in intact brain is unknown, neurotransmitters can have marked effects on CBF[111] followng breakdown of the BBB. We have demonstrated that missile trauma breaks down the BBB. The significant hyperglycemia we have also documented after wounding suggests a massive systemic rise in catecholamines consequent to the missile injury. Systemic NOR and 5HT, therefore, may leak across the disrupted BBB around the missile track and cause CBF changes. Furthermore, abnormal 5HT levels have been implicated in the pathogenesis of cerebral edema following cerebral concussion[82].

We believe that the cerebral "trauma" consequent to the missile injury cannot be satisfactorily studied showing changes in a single physiological measurement e.g., ICP, although this is an important response. Our proposal therefore includes study of brain energy metabolites, neurotransmitter changes, CBF and the BBB which are all interrelated and almost certain to be influenced by missile damage.

Methods An important finding from our studies so far has been the observation of effects distal to the focal cerebral hemisphere wound created by the missile itself. These effects are both anatomic (petechial hemorrhages) and physiologic (bradycardia, hypertension, and "brainstem effects"). Because of these distal effects, tissue sampling techniques which do not encompass the whole brain may miss critical areas of dysfunction. For our proposed studies of metabolism, neurotransmitters, BBB and CBF we intend to use fluorescence photography and autoradiology which will allow us to survey the entire brain.

Method We will anesthetize and prepare the cats according to the General Preparation scheme noting whether the cats will be acute (to 24 hrs) or longer term (21 d). All will be wounded at LD_{50}J energy. Experimental groups and sacrifice times are as indicated:

		Untreated		Treated	
	Normotensive	Non-apneic	apneic	Non-apneic	apneic
	1 hour				
time	6 hours				
after	24 hours				
wounding	21 days				
	Hypotensive				
	1 hour				
time	6 hours				
after	24 hours				
wounding	21 days				

Every time point in each study group requires 5 cats (N = 100). We will, thus, assess acute and sub-acute changes in both untreated and treated normo and hypotensive cats. Six sham-operated cats will be used for controls.

A single cat may be used for both metabolic and neurotransmitters studies because they can be assessed in the same brain slices. Cats for these studies will have their brains frozen in in situ by liquid nitrogen. We will slice the skull and brain with a precooled saw into 1 cm thick slices which will be placed into 5 mm bath of liquid nitrogen. Fluorescence photography will be used to localize areas of cerebral metabolic changes consequent to wounding as indicated by means of NADH in frozen brain slices. Once localized, specific brain slice areas will be sampled and analyzed for NADH, ATP, Pcr and lactate. Adjacent areas will be assessed for Na, K and water. Samples will be made by a cooled 4 mm cork borer adjacent to the main missile track and distally where NADH changes indicated metabolic dysfunction. Portions of frozen brain will similarly be taken for determinations of DA, 5HT, NOR, HVA, DOPAC, 5HIAA and MHPG by high performance liquid chromatography. For both metabolic and neurotransmitter studies similar brain areas will be sampled and comparisons made by AOV to evaluate temporal changes in substrate levels.

Blood may interfere with NADH determinations around the wound track or over the surface of the brain. In general, however, most deeper brain structures not immediately adjacent to the wound track are bloodless. The fluorescence-metabolic studies we propose have been described by Welch[112].

Blood-Brain Barrier, Cerebral Blood Flow, Histology

BBB integrity and CBF measurements will be by standard double labelled autoradiography techniques using [131I]-Iodoantipyrine and [14C]-Sucrose. An important advantage of autoradiography is that it allows detailed profiles to be made around cerebral lesions which could not be obtained by hand dissection. [14C]-Sucrose has been widely used to localize areas of breakdown of the BBB as it is known to have low permeability characteristics in nontraumatized brain. Of particular interest is that its molecular weight (342) and permeability across the BBB is similar to that of physiologically important substrates, e.g. NOR and 5HT.

Preparation wounding and timing for these experiments have been given above. Appropriate intraarterial catheters will be required for blood sampling and all cats will be anesthetized with Brevital during isotope infusions and decaptitation.

Isotope infusions and autoradiographic procedures

Ninety seconds prior to termination of the experiment, [14C]-sucrose (100 μCi/kg) will be infused (IV) so that a rapid rise in the isotope is obtained in the plasma followed by an approximately steady level. Thirty seconds prior to decapitation [131I]-iodoantipyrine (100 μCi/kg) will be injected at a rate of 1.0 ml/min (Harvard Pump). Arterial blood will be sampled immediately following [14C]-sucrose injection into a syringe withdrawn at a constant rate to obtain mechanical integrals of the plasma isotopes[113]. Plasma samples will be counted by liquid scintillation after addition of 1 ml Protocol and 15 ml Aquasol 2. Following decapitation, brains will be rapidly removed and frozen in freon which is liquified by chilling in liquid nitrogen to -40°C. Double label quantitative autoradiographic procedures as previously described will be employed[114]. Sections will also be taken for histological preparation with either hematoxylin and eosin or cresyl violet.

The volume of distribution of sucrose (μl blood/g) = (dpm/g)/(d.p.m./μl)

In brain areas which show an intact blood-brain barrier as indicated by [14C] sucrose measurements CBF estimates are obtained by

$$CBF = \frac{[131I]\text{ brain region}}{\text{mg brain}} \times \frac{\text{syringe withdrawal rate}}{[131I]\text{ blood}}$$

The [131I]-iodoantipyrine concentration in brain tissue will be corrected for activity in blood remaining in the brain tissue by subtracting a background value:

Background d.p.m. = (ml blood/g) x (d.p.m./ml blood).

Data Analysis The values for the volume of distribution of sucrose and CBF for different brain regions in control and brain missile wounded cats (untreated and treated) will be compared by AOV. Differences will be determined by least significant difference.

REPETITIVE MONITORING

Rationale The methods described so far in this proposal require the interrelationships between the neurological status of the animal following brain missile wounding to be made with either, (a) the detailed acute physiological changes (up to 6 hours) or (b) have necessitated the killing of the animal (cerebral trauma studies) for subsequent brain biochemical analysis. It would clearly be important to be able to make direct correlations between the pathophysiological status of the wounded animal (e.g., changes in plasma glucose, central amine metabolism EEG) with the neurological deficits observed in the same animal for longer periods of time. We propose to use repetitive monitoring techniques including implanted blood and CSF catheters and power spectral EEG in order to make these more direct associations. The blood catheter will enable repeated sampling for analysis of amino acids, catecholamines, glucose and lactate. The cisternal catheter will enable repeated measurements of CSF amino acid, 5HIAA, HVA, DOPAC, MHPG, glucose and lactate. EEG is an important way of evaluating brain function and power spectral analysis allows alterations of various frequencies to be quantitated. The temporal pattern of changes in plasma, CSF, substrates and EEG will be correlated with the neurological deficits, observed following wounding for 21 days in both untreated and treated brain missile wounded cats.

Methods Animals will be anesthetized as descibed previously (page 22). A femoral arterial catheter[115] will be exteriorized and a PE10 catheter implanted in the cisterna magna[96,116]. EEG recording electrodes will be placed in the skull and animals allowed to recover for seven days prior to further experimentation. Following this period animals will be anesthetized with Fluothane N_2O/O_2 at 70/30 and wounded using sterile procedures. Samples of blood (2 ml) and CSF (50 µl) will be taken for subsequent biochemical assays. These blood samples and EEG analysis on 10 second samples will be done 1 hour before wounding, then at 1 hr, 6hr, 24hr, and daily (up to 21 days) following brain missile injury. After 1 hour the animals will be awake during EEG analysis. Animals will also be scored for their neurological response (page 24). The following experimental groups will be done, 10 cats per group: control (normotensive); control (hypotensive); normotensive injured at 1.35 J; (untreated and treated); hypotensive injured at LD_{50}J; (untreated and treated).

Data Analyses

Temporal changes in each physiological function will be plotted and correlations made between these and the neurological scores obtained for each cat. Behavioral results will be analysis using the Kruskal Wallis AOV followed by the Mann Whitney U-test to locate specific group differences.

BIOCHEMICAL ANALYSES

The methods for the assay of the different substrates to be measured in blood, brain, and CSF have been previously described. Glucose, lactate, ATP, Pcr, NADH will be determined fluorometrically[117]. AD, NOR, DA, 5HT, 5HIAA, DOPAC, HVA, and MHPG will be determined by isocratic HPLC techniques[116-118]. Amino acids will be determined by a gradient HPLC method[119]. Dr. Sarna has had extensive experience in the use of both fluorometric and HPLC techniques.

FACILITIES

Our laboratory consists of approximately 30 x 45' area divided into one major work area and 5 smaller rooms. Our Dean has promised additional space, if needed. Major equipment includes: physiograph, end-tidal CO_2 monitor, respirator, anesthesia machine, variable velocity helium gun, oven, flame photometer, chloridometer, still, electronic balance, gamma counter, small centrifuge, spectrophotometer, and IBM 9000 computer. We can use a scintillation counter.

LSU has a large Department of Biometry and we will work closely with them for the analysis of the data.

Military Significance

Maximal performance in combat is the top priority of the Army and conservation of the fighting strength is the main job of the Army Medical Corps. The brain wound is the most lethal combat wound and this experimental project directly addresses this critical military problem. Using a standardized, reproducible intracerebral missile wound in an experimental animal, we can screen potentially useful drugs and treatments for the acute care of brain wounds, and correlate these data with the pathophysiology of human brain wounds. This approach offers the most efficient method to lessen mortality, reduce morbidity, and increase the numbers of brain-wounded men able to resume useful Army duty.

Brain damage from a missile is the combat-military equivalent of a cerebrovascular accident or a closed head injury common in civilian life. While these latter conditions have been exhaustively studied, their pathophysiological details and late mechanisms are impossible to transfer to the brain missile wound (see Experimental Background page 11). Details concerning both immediate and subsequent brain pathophysiology following a missile wound to the brain are virtually unknown. This lack has prevented any further improvement in brain wound mortality or morbidity figures since the 1940's and contributes to the loss to the Army of 2 out of every 3 soldiers who survive a penetrating brain wound.

The recent development of computerized tomographic (CT) scans or the new helmet design will probably not have a significant impact on head wound mortality or morbidity because of the following considerations:

(1) In evaluating acute brain trauma, CT scans are extremely effective for diagnosing intracranial bleeding but 90-95% of missile wounds to the brain are associated with only small blood clots (< 20 ml) or slight amount of bleeding. Even if CT scanning might aid diagnosis of brain disruption, it

could not contribute to effective pharmacological treatment of damaged brain to improve residual function.

(2) During Vietnam about 55% of missiles entered the brain by striking below the area of helmet protection.[52] Newer helmet design (reminiscent of the German WWII helmet) increases head coverage somewhat. The German Army during WWII had an excellent helmet and good troop discipline. Despite this, however, German neurosurgeons had to treat many thousands of German brain-wounded casualties.[121]

In any future major war, large numbers of casualties are anticipated and medical care will be limited. If potentially reversible apnea causes death in a significant number of brain wounded persons, as our initial experiments indicate, early life-saving care can be provided at the front by aidmen. If large numbers of soldiers incur brain wounds and neurosurgeons are scarce (and general surgeons are busy treating those with other wounds), definitive treatment will be delayed for many. It would be extremely important to offer these men the most optimal physiologic and pharmacologic treatment to preserve residual brain function, not only for humanitarian reasons but to maximize return to duty.

Table 5 demonstrates the importance of trying to reduce mortality and neurologic morbidity which could significantly contribute to preserving the fighting strength. The figure of 3,000 is used since this was the approximate number of American servicemen who were brain wounded in Korea. The most recent casualty data on brain wounded soldiers in Vietnam showed an acute neurosurgical mortality of 10% with 2 delayed (up to 1 year) additional 7% mortality.[12] In WWII and Vietnam approximately 1 in 3 brain-wounded soldiers returned to duty.

Table 5

Consequences of Decreasing Mortality and Morbidity on Return to Army Duty

(3000 Neurosurgical Brain Wounds)

Mortality	No. Living	Survivors Returning to Army-Various % (33%)	(36%)	(40%)	(50%)
If 17%	2490	822	906	996	1245
if 8.5%	2745	906	988	1098	1373

Assuming the total mortality continues at 17%, if the return to the Army could be increased by only 3% (to 36%), the same number of men (906) would be usefully retained in service as would have been if the mortality had been halved (from 17% to 8.5%). Thus the importance of even a slight improvement in morbidity in conserving the fighting strength is dramatically demonstrated. This argues for concerted attempts to decrease immediate mortality and to maintain and improve the residual function of partially damaged brain.

Currently basic training (13 weeks) for an Army infantryman costs $9,000. Additional specialty training adds to this cost. If our research allows only 170 additional men to return to duty in the course of a war it will have paid for itself. Now the per diem costs at Walter Reed Army Hospital equal $455. Similar per bed day costs may be anticipated for hospitals deployed overseas. If 3000 brain wounded require treatment (as in Korea) this yields ~~a daily~~ care cost of $1,400,000. Research leading to reducing the in hospital days required will save substantial amounts of money for the Army. In a protracted war, the number of brain wounded may be very large. The Germans sustained at least 15-20,000 brain wounded on the Eastern Front alone during WWII.[121]

Use of a laboratory model to screen and evaluate drugs which may decrease mortality and improve brain function after wounding will provide the Army with an efficient means of selecting drugs for future trials in humans. The Army then will know the most worthwhile drugs to use and will be spared the expense of purchasing, storing, replenishing and distributing drugs which are of dubious efficacy for the wounded brain to recover. Financial resources and supply channels can concentrate on drugs of proven worth.

Finally, conservation of manpower and material resources becomes absolutely critical in large scale warfare where neither can be quickly or easily replaced. In this instance, decreasing mortality of the head wound, the most lethal combat wound, becomes critical so that manpower will be available when most needed. We propose to decrease mortality and morbidity through a detailed understanding of the brain wound coupled with the development of optimal physiologic and pharmacologic therapy to improve the residual function of damaged but not destroyed brain tissue.

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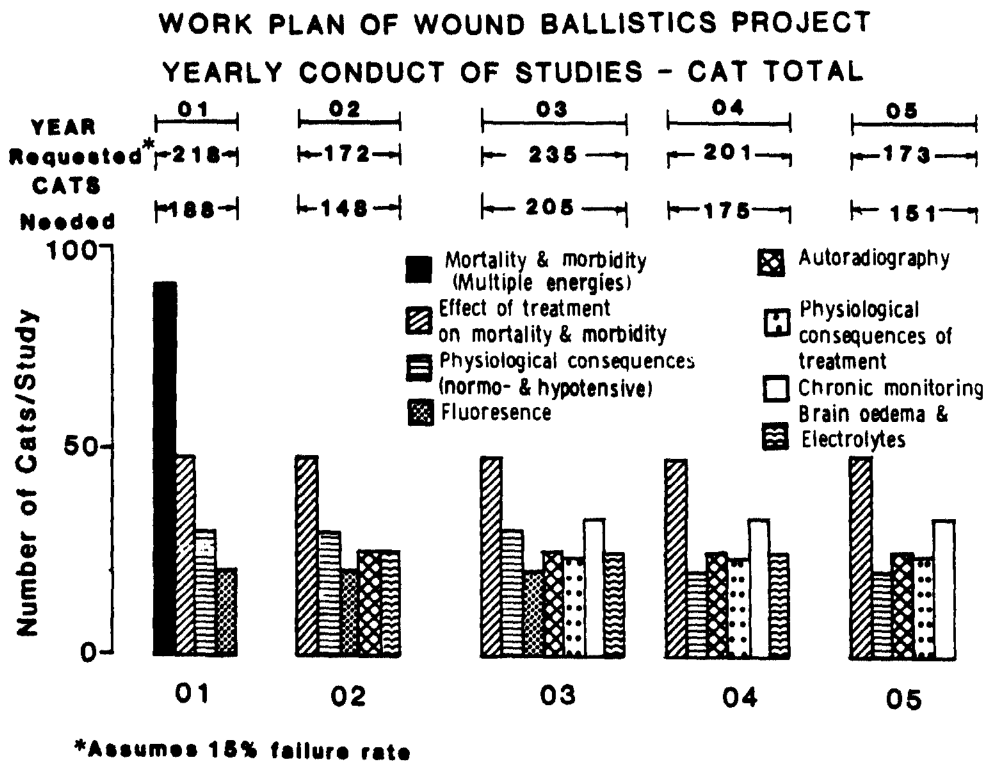

SEF ANIMAL USE DOCUMENTATION (page 83)

RESULTS AND PUBLICATIONS ARISING FROM CURRENT CONTRACT

Owing to two DOD directives to cease ballistic/cat research from September 83 - Jan 84 and Oct 84 - Jan 85, we have had only 8 months to carry out our research. After recruiting and having Dr. Sarna join us in March, we started literally from ground zero, beginning ballistics experiments in March 84 using the helium gun designed by Mr. Robert Carpenter. The initial gun design was faulty, producing very inaccurate shots with variable velocities at the same shooting pressures. Redesign and remanufacture of the gun was completed only in September 1984 because the barrel liner manufacturer would not do the job until July/August 1984. The gun is now very precise, with accurate velocities. Because of inaccuracies, we believed that worthwhile ballistics experiments could begin only after we solved the gun problems.

Despite the short time we have been able to to perform actual experiments, the accomplishments listed in Experimental Background have been achieved; one of the most significant is our development of an experimental ballistics gun which other laboratories can begin to use. (Dr Feurstein from USUHS will shortly be visiting our laboratory and may use a copy of our gun in his laboratory). We will be in a position to publish a paper on the gun design in the next few months pending discussions with Mr. Carpenter.

We have preliminary data on brain swelling and electrolytes following wounding (Experimental Background No 4) but this study will not be complete until March/April 85.

We have presented preliminary data at the NATO meeting in Bethesda, Maryland, Oct 84, and our brain edema data have been accepted for presentation at the 5th International Ballistics Symposium, Gothenberg, Sweden, June 1985.

CURRICULUM VITAE

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RESIDENCE:	[Deleted by GAO.]
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BORN:	[Deleted by GAO.]
MARITAL STATUS:	[Deleted by GAO.]
DEGREES:	A.B. - Yale College, New Haven, Connecticut, 1956 M.D. - Cornell University Medical College, New York City, 1960 M.S. - (Neurosurgery) University of Minnesota, Minneapolis 1970
INTERNSHIP:	University of Minnesota Hospitals, 1-7-60 to 30-6-61 (General Surgery)
RESIDENCY:	University of Minnesota Hospitals, 1-7-61 to 30-6-62 (General Surgery) University of Minnesota Hospitals, 1-7-62 to 30-6-67 (Neurosurgery) Mayo Clinic, Rochester, Minn. 1-1-65 to 30-6-65 Rotation from University of Minnesota
LICENSURE:	Connecticut, Louisiana
SPECIALTY BOARDS:	American Board of Neurological Surgery, 1970
PRIVATE PRACTICE:	Hartford, Connecticut, 1967-1968
ARMY:	Commanding Officer, 378th Medical Detachment (KE) and Chief of Neurosurgery, 312th-91st Evaucation Hospitals, Chu Lai, Republic of Vietnam 1968-1969. Chief of Neurosurgery, William Beaumont General Hospital, El Paso, Texas, 1969-1970 Colonel, U.S.. Army Reserve (MC) 1978 to present "A" designation.
FACULTY APPOINTMENTS:	Consultant of Neurosurgery University of Connecticut, 1967-1968 Assistant Professor of Surgery/Neurosurgery, Louisiana State Medical Center 1970-1974. Associate Professor of Surgery/Neurosurgery, Louisiana State University Medical Center, 1974-1978 Professor of Neurosurgery

Louisiana State University Medical Center, 1978 to present

AWARDS: Army- Bronze star (Service)
Purple Heart
Vietnam Technical Service Medal
Army Commendation Medal

GRANTS:
1. N.I.H. Grant #NS11647-04 with Charles I. Berlin, Follow-up studies on selected men who sustained a brain wound in Vietnam, 1977 to 1983 (approximately $40,000)
2. Bell Award Louisiana Heart Association 1981-1982 Cerebral blood flow and water extraction in rats. ($10,000)
3. U.S. Army Contract # DAMD17-83-C-3145 1983-1985 Physiological Effects of an Experimental Missile Wound in Cats ($300,000)

ORGANIZATIONS: Congress of Neurological Surgeons
American Association of Neurological Surgeons
Society of University Neurosurgeons
Neurosurgical Society of America
Southern Neurosurgical Society
Louisiana Neurosurgical Society-President 1982-1983
Fellow, American College of Surgeons
Louisiana Medical Society
American Medical Association
Royal Society of Medicine, London, England
Founder Member, International Society of CBF and Metabolism

MASTER'S THESIS: (1970) Brain Abscesses at the University of Minnesota Hospitals, 1946-1965

SABBATICAL:
(August 1978- August 1979)
King's College, London with Professor Hugh Davson working on:
1) Effect of hypoglycemia upon cerebrospinal fluid production, iodide clearance and brain electrolytes
2) Amino acid clearance from cerebrospinal fluid

HOSPITAL STAFFS: Charity Hospital, New Orleans, Louisiana
Southern Baptist Hospital, New Orleans, Louisiana
Hotel Dieu Hospital, New Orleans, Louisiana

EXAMINER, NEUROSURGICAL BOARDS:
September, 1980

VISITING PROFESSOR: University of Kentucky, Lexington, Ky

PUBLICATIONS

War Neurosurgery

1. Carey ME, Young HF, Mathis JL, Forysthe J: A bacteriological study of craniocerebral missile wounds from Vietnam. J Neurosurg 34:145-154, 1971

2. Carey ME, Young HF, Mathis JL: The bacterial contamination of indriven bone fragments associated with craniocerebral missile wounds in Vietnam. Mil Med 135:1161-1165, 1970

3. Carey ME, Young HF, Mathis JL: The neurosurgical treatment of craniocerebral missile wounds in Vietnam. Surg Gynec Obstet 135:386-390, 1972.

4. Carey ME, Young HF, Mathis JL: The neurosurgical treatment of craniocerebral missile wounds in Vietnam. An analysis of 224 Vietnamese sustaining brain wounds. The Vietnam Military Medical Journal 40:25-36, 1972

5. Carey ME, Young HF, Mathis JL: The outcome of 89 Americans and 224 Vietnamese sustaining brain wounds in Vietnam. Mil Med 139: 281-284, 1974

6. Carey ME, Young HF, Rish BL, Mathis JL: Late mortality and morbidity observed in a group of 102 American soldiers with a brain wound operated upon in Vietnam. Neurology (Minn.) 24: ,1974

7. Carey ME, Young HF, Rish BL, Mathis JL: A follow up study of 103 American soldiers who sustained a missile wound in Vietnam. J Neurosurg 41:542-549, 1975

8. Carey ME: Invited comment on paper by: Rish BL, Caveness WF, Dillion JD, Kistler JP, et al. : Analysis of brain abscess after penetrating craniocerebral injuries in Vietnam. Neurosurg 9:535-541, 1981

9. Carey ME, Sacco W, Merkler J: Analysis of fatal and non-fatal head wounds incurred during combat in Vietnam by U.S. forces. Acta Chir Scand 508: (Wound Ballistics Fourth International Symposium) 351-356, 1982

10. Carey ME, Tutton RH, Strub RL, Black FW, Tobey EA: The correlation between surgical and CT estimates of brain damage following missile wounds. J Neurosurg 60:947-954, 1984

11. Carey, ME: Combat medical statistics: can they be used to evaluate combat medical care? Learning about and from combat mortality and morbidity data. (submitted to Military Medicine)

PUBLICATIONS

Neurosurgical Infections

1. Carey ME, Chou SN, French LA: Long term neurologic residua in patients surviving brain abscess with surgery. J Neurosurg 34:652-656, 1971

2. Carey ME, Chou SN, French LA: Brain abscesses seen at the University of Minnesota Hospitals 1946-1965. J Neurosurg 36:1-10, 1972

3. Carey ME, Chou SN: Brain Abscess in Conn. HF (ed): Current Therapy , Philadelphia, WB Saunders Co., 1974

4. Carey ME, Chou SN: Infections of the brain, meninges and skull, in Practice of Surgery, Hagerstown, Md., Harper and Row Publishers Inc., 1977

5. Carey ME: Neurosurgical infections, in Howard, RJ and Simmons RL (eds): Surgical Infectious Disease New York, Appleton Century-Crofts, 1981

6. Carey ME, Brain Abscesses in Contemporary Neurosurgery 3:1-5, 1982

7. Carey ME: Infectious diseases in (ed) Rosenberg RN: The Clinical Neurosciences Chapter 10, New York, Churchill Livingstone, 1983

8. Carey, ME: Infections of the central nervous system. (In press, Harper & Row)

9. Carey, ME: Treatment of brain abscesses: Current Therapy in Neurosurgery (In press)

PUBLICATIONS

Physiology

1. Carey ME, Vela AR: The effect of arterial hypotension upon the rate of cerebrospinal fluid formation in dogs. _J Neurosurg_ 41:350-355, 1974

2. Vela AR, Carey ME, Thompson BM: Further data on the acute effect of intravenous steroids on canine CSF secretion and absorption. _J Neurosurg_ 50:477-482, 1979

3. Roheim PS, Carey ME, Forte T, Vega GL: Apoliopoproteins in human cerebrospinal fluid. _Proc Nat Acad Sci_ 76:4646-4696, 1979

4. Carey ME, Davson H, Bradbury MWB: The effect of acute hypoglycemia upon cerebrospinal fluid production, iodide clearance and brain electrolytes in the rabbit. _J Neurosurg_ 54:370-379, 1981

5. Carey ME, Davson H, Bradbury MWB: Effect of severe hypoglycemia upon cerebrospinal fluid production, iodide clearance and brain electrolytes in rabbits (with preliminary observations on the penetration of insulin into CSF) in Cervos-Navarro J, Fritschka, E., (eds): _Cerebral Microcirculation and Metabolism_ New York, Raven Press, 1981

6. Davson H, Hollingsworth JG, Carey ME, Fenstermacher JD: Ventriculo-cisternal perfusion of twelve amino acids in the rabbit. _J Neurobiol_ 12:293-318, 1982

PUBLICATIONS

Others

1. Carey ME, Nance FC, Kirgis HD, Young HF, Megison L, Kline DG: Pancreatitis following spinal cord injury. J Neurosurg 47:917-922, 1977

2. LaCour F, Trevor R, Carey ME: Arachnoid cyst and associated sub-dural hematoma. Arch Neurol 35:84-89, 1978

3. Schecter FG, Carey ME, Bryant LR: Bilateral apical intrathoracic masses associated with Von Recklinghausen's disease. Chest, 75:367-368, 1979

4. Carey ME: Brain Trauma in Practice of Medicine, Hagerstown Md., Harper and Row, Publishers, Inc., 1979

5. Correa AJE, Rodriguez M, Carey ME: SIADH after subarachnoid hemorrhage and craniotomy. South Med J 73:932-934, 1980

6. Carey ME: Brain Trauma in Spittell JA, Jr. (ed) Clinical Medicine Philadelphia, Harper and Row, Publ., 1981 (Chapter 26)

7. Carey, ME: Treatment of gunshot wounds. Current Therapy in Neurosurgery (In press)

Books in Preparation

1. War Neurosurgery (for Surgeon General, US Army)
2. Oral interviews with War Neurosurgeons and Neurologists

Abstracts

1. Carey ME, Vela AR: The effect of multiple levels of arterial hypotension upon the rate of cerebrospinal fluid formation in dog. Fed Proc 33:360, 1974

b

2. Vela AR, Corales RL, Carey ME: The effect of cerebral venous drainage obstruction upon cerebrospinal fluid accumulation. Fed Proc 34:397, 1975

3. Vela AR, Carey ME, Thompson BM: The effect of dexamethasone on canine cerebrospinal fluid production. Fed Proc 35:268, 1976

4. Fritschka E, Carey ME, Vela AR, Spitzer JJ: Effect of insulin induced hypoglycemia on cerebrospinal fluid production. Dept. of Physiol and Neurosurg, L.S.U. M.C. Sch., New Orleans, La. Society for Neurosciences, 1977

5. Vela AR, Carey ME, Walker K: The effect of hypotension upon ventricular absorption of phenosulfonphtalein. Fed Proc 36:570, 1977

Talks Presented

1. Bacteriology of War Wounds: Gary Wratten Symposium, 1970, Walter Reed Institute of Research, Washington, D.C.

2. Bacteriology of War Wounds: Congress of Neurological Surgeons, 1970.

3. Mortality and Morbidity Associated with Craniocerebral Missile wounds in Vietnam, Gary Wratten Symposium, 1971, Walter Reed Institute of Research, Washington, D.C.

4. Mortality and Morbidity Associated with Craniocerebral Missile Wounds in Vietnam, Southern Society of Clinical Surgeons, 1971.

5. Mortality and Morbidity Analysis of 91 American Soldiers with Intracerebral Wounds: Congress of Neurologic Surgeons, 1971.

6. Intermediate Follow Up on 89 American Soldiers who Sustained Intracerebral Missile Wounds in Vietnam. Gary Wratten Surgical Symposium. Walter Reed General Hospital, Washington, D.C., 1972.

7. Intermediate Follow Up on 89 American Soldiers Who Sustained Intracerebral Missile Wounds in Vietnam. Congress of Neurological Surgeons, Post Convention Meeting, Colorado Springs, Colo., 1972.

8. The Effect of Hypovolemic Hypotension on Cerebrospinal Fluid Formation in the Dog. Association for Academic Surgery, New Orleans, La. 1972.

9. The Effect of Systemic Arterial Hypotension Upon the Rate of Cerebrospinal Fluid Production in Dogs. American Association of Neurological Surgeons, Los Angeles, California, April 1973.

10. Neurologic Disabilities in Brain Injured Soldiers: A Three Year Follow Up. American Academy of Aphasia. Albaquerque, New Mexico, October 1973.

11. Late Mortality and Morbidity Observed in a group of 103 American Soldiers with a Brain a Wound Operated Upon in Vietnam. Southern Neurosurgical Society, Key Biscayne, Fla., February, 1974.

12. The Influence of Several Levels of Hypovolemic Hypotension Upon the Rate of CSF Formation in the Dog. American Association of Neurologic Surgeons, St. Louis, Missouri, April 1974.

13. Current Concepts in Cerebral Spinal Fluid Physiology. American Association of Neurological Surgeons, Miami, April 1975.

14. Head Trauma. American Association of Neurological Surgeons, San Francisco, California, April 1976.

15. Spinal Cord Injury and Pancreatitis. American Association of Neurological Surgeons, San Francisco, California, April 1976.

16. Vertebral Osteomyelitis. American Association of Neurological Surgeons, Miami, April, 1975.

17. Prophylactic Antibiotics in Neurosurgery. American Association of Neurological Surgeons, New Orleans, Louisiana, April, 1978.

18. Treatment of Brain Abscess (Seminar). American Association of Neurological Surgeons, Los Angeles, California, April 1979.

19. Comments on the Production of Experimental Brain Abscess. American Association of Neurological Surgeons, New York, N.Y., April 1980.

20. Effect of Severe Hypoglycemia on CSF Formation, Ventricular Iodide Clearance and Brain Electrolytes. Erwin Riesch Symposium, Berlin, Germany, July, 1980.

21. War Medicine and Combat Neurosurgery. Invitation of Surgeon General, US Air Force; Wilford Hall Hospital, San Antonio, Texas, March 1981.

22. Neurosurgery in Vietnam, Uniformed Services Medical School, Bethesda, MD., Bethesda, MD., October 1981.

23. Treatment of Brain Abscess. American Association of Neurological Surgeons, Boston, Mass., April 1981.

24. War Medicine and Combat Neurosurgery. Invitation of Surgeon General, US Air Force, Munich, Germany, August, 1981.

25. An Analysis of Fatal and Non-fatal Head Wounds Incurred during Combat in Vietnam by US Forces. 4th International Ballistics Symposium Gothenberg, Sweden, September, 1981.

26. War Medicine and Combat Neurosurgery. Invitation of Surgeon General, US Air Force Andrews AFB, MD, October, 1981.

27. Late CT Findings Following Missile Wounds to the Brain. Universitats Klinik, Koln, Germany, September, 1983

28. Late CT Findings Following Missile Wounds to the Brain. Universitats Klinik, Essen, Germany September 1983

29. Neurosurgical Considerations of Missile Wounds to the Brain. Gunzburg, Germany, September 1983

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ADDRESS (home): [Deleted by GAO.]

EDUCATION :

School: (a) Tottenham Grammar School (1963-1965)

(b) Mill Hill School (1965-1969)

University: (a) Manchester University (1970-1973)

Department of Physiology

B. Sc. (Hons) Physiology- 2 (1)

(b) London University (1974-1978)

Department of Physiology,

Kings College, Strand, London WC2

Ph.D. Physiology (1978)

Thesis title: Studies on the blood-brain barrier after portacaval anastomosis in the rat

Supervisor: Professor M.W.B. Bradbury

POST-DOCTORAL POSITIONS :

(a) Member of the non-clinical scientific staff at the Medical Research Council, Toxicological Unit, Carshalton, Surrey. (1978-1980)

Head of Section: Dr. J. Cremer

(b) Research Fellow at the Department of Neurochemistry, Institute on Neurology, London WC1 (1980-1984)

Head of Section: Professor G. Curzon

(c) Assistant Professor in Neurosurgery and Physiology Department of Neurosurgery, LSU Medical Center, 1542 Tulane Avenue, New Orleans, LA 70112 (1984- present)

Head of Section: Dr. M. Carey

TEACHING EXPERIENCE :

Demonstrations etc. to 1st, 2nd and 3rd year B.Sc. students. Involved in organising and running 3rd year Mammalian Physiology Course (Department of Physiology, Kings College). Lectures to M. Sc. students in Neurochemistry (Institute of Neurology).

SOCIETY MEMBERSHIP :

Physiological Society (London)

Royal Society of Medicine

REFEREE EXPERIENCE :

I have refereed project grant applications for the Medical Research Council and papers for the Journal of Neurochemistry, Clinical Science, Brain Research and Gut.

BIBLIOGRAPHY

PAPERS: Published

Sarna, G.S., Bradbury, M.W.B. & Cavanagh, J.B. (1977) Permeability of the blood-brain barrier after portocaval anastomosis in the rat. Brain Res., 138, 550-554

Sarna, G.S., Bradbury, M.W.B., Cremer, J.E., Lai, J.C.K. & Teal, H.M. (1979) Brain metabolism and specific transport at the blood-brain barrier after portocaval anastomosis in the rat. Brain Res., 160, 69-83

Cunningham, V.J. & Sarna, G.S. (1979) Estimation of the kinetic parameters of unidirectional transport across the blood-brain barrier after portocaval anastomosis in the rat. J. Neurochem., 160, 69-83

Cremer, J.E., Cunningham, V.J., Ray, D.E. & Sarna, G.S. (1980) Regional changes in brain glucose utilisation in rats given a pyrethroid insecticide. Brain Res., 194, 278-282

Milligan, S.R.M. & Sarna, G.S. (1981) Effect of portocaval anastomosis and chronic underfeeding on the hypothalamic pituitary-gonadal axis in the rat. J. Endocr., 88, 39-47

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Cremer, J.E., Ray, D.E., Sarna, G.S. & Cunningham, V.J. (1981) A study of the kinetic behaviour of glucose on stimultaneous estimates of influx and phosphorylation in brain regions of rats on different physiological states. Brain Res., 221, 331-342

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Sarna, G.S., Hutson, P.H., Tricklebank, M.D. & Curzon, G. (1983) Determination of 5-hydroxytryptamine turnover in freely moving rats using repeated sampling of cerebrospinal fluid. J. Neurochem., 40, 383-388

Hutson, P.H., Sarna, G.S., Kantamaneni, B.D. Curzon, G. (1984) Concurrent determination of brain dopamine and 5-hydroxytryptamine turnovers in individual freely moving rats using repeated sampling of cerebrospinal fluid. J. Neurochem. 43, 151-159

Hutson, P.H., Sarna, G.S. & Curzon, G. (1984) Determination of daily variations of brain 5-hydroxytryptamine and dopamine turnover and of the clearance of the acid metabolites in conscious rats by repeated sampling of cerebrospinal fluid. J. Neurochem., 43, 291-293

Sarna, G.S., Hutson, P.H. & Curzon, G. (1984) Effect of α- methyl-fluorodopa on dopamine metabolites: importance of conjugation and egress. Europ. J. Pharmacol. 100: 343-350

PAPERS: In Press

Sarna, G.S., Kantamaneni, B.D., & Curzon (1985) Variables influencing the effect of diet on brain tryptophan. J. Neurochem.

Hutson, P.H., Sarna, G.S., Kantamenni, B.D. & Curzon, G. (1985) Monitoring the effect of a tryptophan load on brain indole metabolism in freely moving rats by simultaneous cerebrospinal fluid sampling and brain dialysis. J. Neurochem.

PAPERS: Submitted for publication

Honig, A., Sarna, G., Bouras, N., Curzon G., Bridges, P.K. & Barlett, J.R. (1984) Plasma, CSF and brain concentrations of GABA and other amino acids in depressive illness. Submitted for publication

Curzon G, Hutson, PH, Kantameni B.D., Sahakian B.J. & Sarna GS (1985) Dopamine and 5-hydroxytryptamine metabolism in the rat: acid metabolism in cisternal cerebrospinal fluid before and after giving probenecid. Submitted for publication. J. Neurochem.

Sahakian B.J., Sarna, G.S., Kantamaneni, B.D., Jackson, A., Hutson, P.H. & Curzon, G. (1985) Association between learning and cortical catecholamines in non-drug treated rats. Submitted for publication. J. Neurochem.

Pinkerton, C.R., Smith I, Leeming R.T., Sarna, G.S., Hyland, K., Curzon, G., & Chessells, J.M. (1984) Methotrexate therapy is not associated with neuro-transmitter amine deficiency. Submitted for publication. Arch. Dis. Child-hood

Prasad, C., Edwards, R & Sarna, G.S. (1985) Differences in the properties of pyroglutamate aminopeptidases from rat cerebrospinal fluid, brain and other tissues. Submitted for publication. J. Neurochem.

PAPERS: In Preparation

Sarna, G.S. & Bradbury, M.W.B. (1985) Effect of portacaval anastomosis in the rat on brain electrolyte and water content.

Sarna, G.S., Gwilliam,R. & Cremer, J.E. (1985) Simple devices for regional dissection of microwaved rat brain and repeated sampling of blood from the conscious rat.

Sarna, G.S., Green, P., Tricklebank, M.D. & Curzon, G. (1985) An in vivo approach to investigate transport processes beyond the blood-brain barrier.

Sarna, G.S., Hutson, P.H., Cunningham, V.J., Bradbury, M. & Curzon, G. (1985) Brain intracellular and extracellular concentrations of tryptophan, di-hydroxyphenylacetic acid, homovanillic acid and 5-hydroxyindoleacetic acid using the dialtrobe.

REFFERED COMMUNICATIONS

Cremer, J.E., Lai, J.C.K. & Sarna, G.S. (1977) Rapid blood-brain transport and metabolism of butyrate and pyruvate in the rat after portocaval anastomosis. J. Physiol. (London), 266, 70p

Bradbury, M.W. and Sarna, G.S. (1977) Portocaval anastomosis in the rat- some effects on the brain and elsewhere. J. Physiol. (London), 266, 26P

Curzon, G., Hutson, P.H., Sarna, G.S. & Tricklebank, M.D. (1982) Determination of brain 5-hydroxytryptamine turnover in freely moving rats using repeated sampling of cerebrospinal fluid. Brit. J. Pharm., 77, 311P

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BOOK CHAPTERS

Bradbury, M.W.B. & Sarna, G.S. (1977). Homeostatis of the ionic composition of the cerebrospinal fluid. Exp. Eye Res., 25, 249-258

Cremer, J.E., Sarna, G.S., Teal, H.M. & Cunningham, V.J. (1978) Amino acid precursors: their transport into brain and initial metabolism: in Amino Acids as Chemical Transmitters, A 16: pp 669-689 Ed. F. Fonnum, Plenum Press.

Curzon, G., Hutson, P.H., & Sarna, G. (1983) Concurrent determination of central dopamine and 5-hydroxytryptamine turnover in the conscious rat using CSF sampling. Prog. Neuropsychopharmacol. Suppl. 107

Hutson, P.H. & Sarna, G.S. (1983) Monitoring transmitter metabolism in the living brain. Biochemistry of the Nervous system. Eds. A.T. Patel & A.N. Davison. Published by the Biochemical Society.

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Curzon, G., Hutson, P.H., Jackson, A., Sahakian B.J. & Sarna, G. (1985) Monitoring brain amine metabolism using CSF: validation of method and use in the investigation of relationships with social behaviour. In Monitoring Peripheral and Central Neurotransmitter Release To be published.

ABSTRACTS

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Curzon, G., Hutson, P.H. & Sarna, G.S. (1983) The removal of metabolites via the CSF as an index of central transmitter amine metabolism Abst. 3rd Int. Meeting on Tryptophan Metabolism (Evian)

Curzon, G., Hutson, P.H. & Sarna, G.S. (1983) Concurrent determination of central dopamine and 5-hydroxytryptamine turnover in the conscious rat using CSF sampling. 5th Int. Catecholamine Symposium. Goteburg

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Sahakian, B.J., Sarna, G.S., Jackson, A., Hutson, P.H. & Curzon, G. (1984) Application of an vivo monitoring of CSF transmitter amine metabolism in behavioural studies. 14th CINP Congress Florence, Italy, p634

Curzon, G., Hutson, P.H., Kantamaneni, B.D., Sarna, G.S. (1984) Monitoring effects of drugs on brain indole metabolism by repetitive withdrawal of CSF from freely moving rats. IUPHAR 9th International Congress of Pharmacology.

Hutson, P.H., Sarna, G.S., Sahakian, B.J., Jackson, A. & Curzon, C. (1984) Use of repeated sampling cisternal CSF in the study of transmitter amine metabolism and behaviour of conscious, freely moving rats. 4th European Winter Conference on Brain Research.

Hutson, P.H., Sarna, G.S. & Curzon, G. (1984) What do CSF dopamine metabolites tell us about brain metabolism? Symposium: Monitoring Peripheral and Central Neurotransmitter Release (Oxford).

Curzon, G., Hutson, P., Jackson, A., Sahakian, B.J. & Sarna, G.S., (1984) Monitoring brain amine metabolism using CSF: Correlations with behaviour. Symposium: Monitoring Peripheral and Central Neurotransmitter Release (Oxford)

Pinkerton, C.R., Smith, I., Leeming, R.J., Curzon, G., & Sarna, G. (1984) Are cerebrospinal neurotransmitters related to neurotoxicity in children receiving treatment for acute lymphoblastic leukaemia? Brit. Ass. Cancer Res.

Carey, M.E., Farrell, J.B. & Sarna, G.S. (1984) Pathophysiological effects of missile wounding in the cat. NATO Defense Research Study. Symp: Wounds, wound coverings and wound contaminations.

PROBLEM AREAS

(1) Project cost: While monies requested are substantial, this initial outlay will ultimately save the US Army money. We are taking the most direct, up to date research approach to learn about the number one cause of death from combat wounding. Prudence dictates that brain wounding be studied intensively and definitively for timely investigation of the most appropriate therapy. Our proposal includes study of most of the major areas relating to brain function. Quickly learning how a missile wound causes dysfunction in these crucial areas will most efficiently point the way to future research and effective therapy. Insights gained will prevent the Army from expending funds in non-productive research areas related to brain wounding. Purchase of non-effective drugs will be avoided.

(2) Too diverse: The proposal covers a variety of fields of study, including brain metabolism, CBF, BBB, neurotransmitters and behavior. It may be argued that we are attempting to do too many diverse topics. However, we feel that each aspect of the project is interrelated and merits detailed investigation. Dr. Sarna has an extensive background in each area of study proposed and has published papers in each field (see Curriculum Vitae). Dr. Carey, a neurosurgeon and research scientist, who from his personal experiences in Vietnam has a very practical awareness for the necessity of developing new treatments for brain missile wounded soldiers. We are in a position to apply the knowledge gained from the animal project to the clinical situation.

(3) Level of hypovolemic shock: In the proposal we are considering an experimental group of animals that are hemorrhaged following brain missile wounding. This is to simulate in the laboratory the hypovolemic shock that occurs in conjunction with many brain wounds in combat[58]. It is known that reducing the cerebral perfusion pressure to below 40 mm Hg results in marked changes in brain energy metabolites of non-traumatized animals[120]. We have chosen to reduce the MABP to 40 mm Hg (a cerebral perfusion pressure of approximately 80 mm Hg) for 1 hour and preliminary experiments will determine whether this level of hypovolemic shock is too extreme to get enough animals for the behavioral evaluation.

(4) Multiple drug therapy: Brain missile wounding results in a number of complex pathophysiological changes. We recognize that the most efficacious treatment may not be a single drug but a

combination of drugs with or without respiratory support.

5) Blocked catheters: Repetitive sampling of CSF and blood for up to 21 days following brain missile wounding has been proposed. There many be a number of cats in which the CSF catheter in particular becomes blocked.

6) CSF metabolites: We will be using the levels of 5HIAA, DOPAC and HVA in CSF as indices of serotonergic and dopaminergic function. Most previous studies have only measured the free forms of DOPAC and HVA which are only valid indices if no conjugated forms are present. The proportion of conjugated DOPAC and HVA in cat CSF is unknown. We will determine whether conjugated forms[116] are present and if so use total (free + conjugated) DOPAC and HVA as indices of dopaminergic function.

7) Fluorescence contaminants: We will be using fluorescence photography to determine ischemic brain areas following brain missile wounding as indicated by increases in NADH fluorescence. However it is known that artificially high fluorescence is observed with the presence of hemoglobin in frozen brain slices. This is a potential problem in our animal model as there is blood both focal and distal to the missile tract. However, we are not relying on native fluorescence alone in determining NADH levels but will be taking samples of frozen brain regions for direct biochemical analysis.

8) CBF studies: Accurate determination of CBF using [13I]-iodoantipyrine using autoradiographic procedures requires knowledge of the intravascular space for background correction. Marked alterations in blood volume will influence the CBF values obtained. We will therefore only make detailed CBF measurements in areas shown to have an intact blood-barrier and normal blood volume as indicated by the sucrose studies. Autoradiography is now widely used for the determination of regional cerebral glucose metabolism and has been used in studies of concussive head injury. We do not propose to do glucose utilization studies as it would require too many assumptions to be made, e.g. normal glucose influx and steady state kinetics. The radiolabelled substrates proposed for our autoradiographic studies are either freely diffusible (iodoantipyrine) or relatively impermeable (sucrose) and are not influenced by either facilitated

transport processes or metabolism.

(9) Personnel and Work Load: The wide number of procedures to be employed in the project will require additional personnel. We will have a PhD with a background in biochemistry to establish the blood and brain metabolite assays. This appointment will last 1.0-1.5 years. Following this we will obtain services of a PhD familiar with autoradiography for the proposed CBF and BBB studies. Another new technician will be employed for the duration fo the project. The relatively high rates of salary requested is to enable personnel of high academic merit to be attracted to the project. This will ultimately save the Army money because a higher quality of research will be accomplished in the shortest time.

(10) Future proposals: The techniques to be employed the present proposal can be extended in many ways for future study e.g. 1) autoradiography may be used to localize and characterize specific receptors for neurotranmitters and 2) repetitive monitoring with a dialtrobe would allow determineation of the release of various neurotransmitters which more closely reflect behavioral changes and 3) levels of CSF neurone specific enolase would give direct indices of the extent of brain damage.

(11) Lack of publications from presently funded research: This is entirely because of the lab start-up, initial gun malfunction (now corrected) and DOD "holds" on reseach. Both investigators have, in the past, published articles in referred journals concerning brain electrolytes, our 01 contract obligations. Our current investigations in this area had been proceeding satisfactorily. Now that we can once again do experiments, we anticipate concluding this phase of research in the spring of 1985.

ANIMAL USE DOCUMENTATION

Investigators : Michael E. Carey, M.D., M.S., and George S. Sarna, Ph.D.

Project Title : An Experimental Brain Missile Wound; Ascertaining Pathophysiology and Evaluation of Treatments to Lower Mortality and Morbidity

We will use 3-4kg mongrel cats, delivered to LSU by the USDA licensed dealer whom we have used for the past 1.5 years. We will try to get cats 0.5 to 2.0 years old. Our experiments will require 3-4 cats/week for 2 PhDs; yearly requirements will be 150 to 210 cats per year.

All cats will be quarantined for 2 weeks upon receipt by LSU and given various shots by our in-house veterinarians. We will observe and score their normal behavior during quarantine 2 days directly before experimentation. We will anesthetize all animals for surgery and wounding so they will feel no pain. Anesthesia will consist of IP or IV Brevital(R) or Halothane-N_2O/O_2 70/30 via anesthesia box or endotracheal tube. Local 1% xylocaine anesthesia also will be used in any animals getting N_2O/O_2 alone after halothane induction. All animals will have an endotracheal tube initially. Surgery under anesthesia will consist of one or two 3-4cm groin incisions for arterial and venous catheter placements plus a 4cm scalp incision for removal of the anterior wall of the right frontal sinus. We will perform surgery under clean conditions for our acute cats (to be sacrificed on the same day) and sterilely for longer term cats (to remain alive for 21 days) which we wish to observe and score for behavior after wounding and treatment. We will not allow acute cats to awaken after wounding. After completing the experiment, we will sacrifice them with simultaneous IV barbiturate and decapitation or IV KCL and brain freezing with liquid N_2. We will anesthetize the longer term cats as the acute ones but surgery and wound closure will be sterile. We will wound these cats with a sterile sphere to obviate infection. After wounding and closure of the 4cm scalp wound, we will remove chronic cats from the stereotaxic frame so they will have no pain upon awakening. We will remove the endotracheal tube as soon as safely possible. These wounded animals will be placed in warmed cages in our laboratory for intensive nursing care as needed during their early post-wounding convalescence. Maintenance fluids will be by IP route. We will treat them with penicillin and apply local antibiotic to the wounds. We have already done 2 chronic cats in this fashion (purchased with local, non DOD funds). They seemed to awaken well and did <u>not appear to be in any pain from their minimal surgery or wounding</u> . After 3-4 days even the hemiparetic cat could eat and drink well on his own. Neither brain-wounded animal developed an infection and we could easily observe their neurologic behavior.

We will wish to monitor power spectral EEGs and blood and CSF metabolites for 3 weeks after wounding in certain subacute cats to aid in assessing drug therapy and posible effects of drugs on brain function during the recovery phase. These animals will have a chronic cisternal cannula and 4 skull screws attached to EEG leads from a Harvard infusion-and-electrical-swivel for connection to infusion pumps and EEG. These cats will be restrained as needed in a Harvard sling. These are all standard laboratory designs and procedures which Dr. Sarna has utilized in the rat. Ultimately longer term animals will be painlessly sacrificed with IV barbiturate plus decapitation or brain freezing with liquid N_2.

Both acute and chronic animals must be sacrificed so we can analyze their brains for energy status (NAD/NADH,ATP/Pcr/Lactate), blood flow, blood-brain barrier and neurotransmitters by HPLC.

JUSTIFICATION FOR CATS AS THE EXPERIMENTAL MODEL

We have selected the cat for our experimental animal because it is small, relatively inexpensive and thus a feasible animal for drug screening and detailed physiologic studies. Because of its relatively low weight, isotope doses, while expensive, are not prohibitive (as would be in a 40 pound 6 month old miniature pig). Most importantly, however, the cat has ample grey and white matter as does the human brain. Traumatic brain edema develops primarily in white matter. Smaller animals as rabbits and rats have very little cerebral hemisphere white matter, their mantle consisting primarily of cortical gray. Their brains, therefore, are quite unlike humans. Mongrel dogs have irregular head shapes precluding uniform fixation in the stereotaxic frame and standard wound placement. In doing ballistics work one also has the scaling factor. In Vietnam, the average missile weight causing a fragment wound was 0.1 to 0.2gm. The human brain weighs 1300gm. In our experiments we create a brain wound in a 25gm cat brain with a 0.030gmsphere. Our missile is 8-16 times too heavy, proportionately, but according to Mr. Robert Carpenter, formerly of the Edgewood Arsenal who designed our helium gun, a 0.030gm sphere is the smallest that can be practically fired. Use of a 0.030gm sphere in a smaller animal as rat (1.0gm brain) or rabbit (7.0gm brain) would be quite unrealistic and would not provide appropriate information for transfer to the human situation. Use of the cat represents a compromise between animal size, brain size and configuration, and missile size to obtain the most sophisticated, physiological results possible to elucidate the human brain wound.

To our knowledge we are the first to use cats for experimental ballistics research and, the first to carry out such experiments with a realistically small missile through the intact skull. As long ago as 1942,[121] however, the cat was used to study brain swelling relative to military wounds. In recent years the cat has been extensively used to study brain swelling, brain energy states,[54] blood flow and percussion injury.[57,59,62,63,108,109]

Our 5 year experimental program is outlined in the following table. We have tried to gain appropriate data with the minimal number of cats.

	Number of Cats	Year of Project
I. NEUROLOGICAL STATUS		
(a) Mortality and morbidity (multiple energies)	90	01
(b) Effect of various treatment regimes on mortality and morbidity (1.35J, normotensive cats)	240	01,02,03,04,05
(c) The pathophysiological consequences of the most efficacious treatment(s) will be studied (1.35J, normotensive cats)	72	03,04,05
II. PATHOPHYSIOLOGICAL STATUS		
(a) Acute physiological consequences: CNS and systemic	90	01,02,03
(b) Cerebral trauma: Effects on (i) Regional cerebral energy metabolism	100	01,02,03,04,05
(ii) Neurotransmitter systems (iii) Cerebral blood flow (iv) Blood-brain barrier	100	02,03,04,05
(c) Chronic physiological and neurochemical consequences	100	02,03,04
(d) Determination of cerebral edema and electrolytes	75	02,03,04
SUB TOTAL	867	
Additional cats for failed experiments	143	
TOTAL CATS REQUESTED	1010	

The LSU Animal Facility is staffed by two veterinarians who evaluate and treat all animals assurring their comfort and good health. They are able to diagnose and treat feline diseases. The LSU Animal Facility is fully AAALAC accredited.

In this project we will adhere to all precepts for animal care contained in the Guide for the Care and Use of Laboratory Animals (PHS Pub #80-23, 1980)

Campus Correspondence

LOUISIANA STATE UNIVERSITY
SCHOOL OF MEDICINE

FROM: R.R. Gonzalez, D.V.M.
Animal Care

NO: ____________

TO: Michael Carey M.D.
Neurosurgery

DATE: 1-18-85

This will acknowledge receipt of the Research Summary for your grant proposal entitled:

PHYSIOLOGICAL EFFECTS OF AN EXPERIMENTAL MISSILE WOUND TO THE BRAIN

[XX] Your proposal as written is acceptable to this department. When you submit the final draft of your grant proposal to the Assistant to the Chancellor, please include a copy of this memo.

IMPORTANT: Please inform the Animal Care Department Director immediately upon notification of grant approval and funding. At this time, we strongly recommend that you inform the Animal Care Department of specific details concerning any special care of animals to be used for your research project. Reference No.________

[ ] We have forwarded your proposal to the Chairman of the ________________ Committee for evaluation and study. We suggest that you contact the Chairman of that committee to obtain further details and information. Animal Care approval of grant proposals is subject to approval by the respective Committee.

[ ] The information contained in your proposal should be discussed with the Animal Care Department Director. Please call 568-6090 in order to establish a time and date to discuss this more fully.

R.R. GONZALEZ, D.V.M.
Director, Animal Care Dept.

RRG/lbh

OTHER SPONSORS AND/OR SUPPORT:

We are currently funded by the Army DAMD17-83-C-3145 until 1st October 1985.

Abbreviation	Definition
AA	Amino acids (tryptophan, phenylalanine, tyrosine, methionine, histidine, leucine, isoleucine, valine, threonine, asparagine, serine, proline, glutamine, ornithine, lysine, arginine, glutamate, taurine, alanine, glycine, aminobutyric acid).
ABG	arterial blood gases
AD	adrenaline
AOV	analysis of variance
ATP	adenosine - 5' - triphosphate
BBB	blood-brain barrier
BP	blood pressure
CBF	cerebral blood flow
cc	cubic centimeter
cm	centimeter
CNS	central nervous system
CO	cardiac output
CO_2	carbon dioxide
CSF	cerebrospinal fluid
CT	computerized tomography
DA	dopamine
DOD	Department of Defense
DMSO	dimelthylsulfoxide
DOPAC	di-hydroxyphenylacetic acid
EEG	electroencephalogram
$ETCO_2$	end tidal CO_2

ft	foot
g	gram
GLU	glucose
Hg	mercury
5-HIAA	5-hydroxy-indole-3-acetic acid
HPLC	high performance liquid chromatography
HR	heart rate
HCT	hematocrit
HVA	homovanillic acid
ICP	intracranial pressure
I.V.	intravenous
I.P.	intraperitoneal
J	joule
kg	kilogram
L-dopa	L-dihydroxyphenylalanine
MABP	mean arterial blood pressure
mCi	milli-Curie
µCi	micro-Curie
mg	milligram
MHPG	4-hydroxyphenethyleneglycol
min	minute
ml	milliliter
µl	microliter
mm	millimeter
MW	molecular weight
N_2	nitrogen
Na	sodium

Na/K	sodium potassium ratio
NAD	nicotinamide
NADM	reduced nicotinamide
NATO	North Atlantic Treaty Organization
N_2O	nitrous oxide
NOR	noradrenalin
O_2	oxygen
OSM	osmolarity
$PaCo_2$	arterial partial pressure of carbon dioxide
Pcr	phosphocreatine
PE	polyethylene
PSA	power spectral analysis
THAM	tromethamine
TRH	thyrotropin releasing hormone
USUHS	Uniform Services University of Health Sciences
WDMET	Wound Data and Munitions Effectiveness Team

Description of LSU Research Experiments

The LSU research team provided us with written information on the 33 experiments performed to date. These experiments involved the following:

- blood gas controls,
- electrolytes,
- preinjected Evan's Blue Dye,
- prostaglandins,
- Evan's Blue Dye injected postwounding,
- physiology,
- coagulation factors,
- histology,
- behavior,
- cerebral blood flow,
- apnea,
- plasma catecholamines,
- brain catecholamines,
- recovery,
- glucose catecholamines,
- photog,
- blood contamination,
- audio-evoked potentials,
- pulmonary edema,
- TTC-BBB breakdowns,
- circling (isoflurane),
- left ventricular cannula,
- anesthesia, and
- blood flow in the brain and other body organs following brain missile wounding (10 experiments).

The LSU team's written information was provided, in June 1989, to our panel for its review. In September 1989, we interviewed various members of the LSU research team to clarify and supplement aspects of the written information.

This appendix contains all information we received about the 33 experiments, with, first, a summary of the procedures needed to prepare animals for wounding (anesthesia protocols A and B). Most of the information is quoted from the written description provided by LSU; we have made minor editorial changes to these. All emphases noted were in the original text. We have also included updated information obtained from our September interviews.

Anesthesia Protocols A and B for Research as Performed (Summary of Procedures)

Protocol A: Protocol for Acute Physiologic Experiments

1. The cat is weighed and given the appropriate dose of pentobarbital (approximately 40 mg/kg) via IP injection.

2. The adequacy of the anesthesia is tested using the two following criteria:

a. lack of limb withdrawal from a mild pinch (using index finger and thumb) between the toes; and
b. absence of corneal reflex to touch (tip of paper tissue touched to cornea).

A small (approximately 1/2 inch) incision is made on the right rear leg for cannulation. After treatment of the incision with lidocaine (2%), the femoral artery is cannulated to monitor blood pressure, and the femoral vein is cannulated for anesthetic supplementation. If the cat shows any indication of inadequate anesthesia, it is supplemented with pentobarbital in titrations of 6.5 mg via IV injection.

3. After application of 2% lidocaine to the epiglottis, an endotracheal tube (with 2% lidocaine gel applied to the end) is inserted.

4. The cat is mounted in the stereotaxic frame.

a. An arterial catheter is attached to a pressure transducer, and the endotracheal tube is connected to a CO_2 monitor.
b. Depth of anesthesia is again assessed using the previously described criteria as well as the MABP, end-tidal CO_2, and respiratory rate. All of these factors together are much better indicators than any one alone.
c. Again, anesthesia is titrated in aliquots of 6.5 mg until sufficient dosage is achieved.

5. The surgery is performed:

a. The cat's head is shaved and a 2-inch incision is made midline. The anterior wall of the right frontal sinus is removed.
b. A small (2 mm) burr hole is then made on the left side for insertion of an intracranial pressure (ICP) transducer.
c. The cat is observed for any signs of discomfort and is supplemented as needed through the venous cannula.

6. The missile wound is induced. The cat is given respiratory support as required and is removed from the frame within minutes of wounding. Deep anesthesia is maintained throughout.

Protocol B: Standard Protocol for Cats Intended to Survive and Used for Behavioral and Drug Testing for Research as Performed

1. The cat is weighed, and an appropriate dose (40 mg/kg, IP) of pentobarbital is administered.

2. The weight of the cat is entered into a computerized record.

3. ALL SURGICAL PROCEDURES ARE PERFORMED UNDER STERILE CONDITIONS. [Emphasis in original.]

4. The adequacy of the anesthesia is tested using the two following criteria:

a. lack of limb withdrawal from a mild pinch (using index finger and thumb) between the toes; and
b. absence of corneal reflex to touch (tip of paper tissue touched to cornea).

Once the depth of anesthesia is deemed adequate, one arterial cannula is implanted after treatment of the incision area with local anesthetic (2% xylocaine). IF THE CAT SHOWS ANY SIGN OF DISCOMFORT DURING THE CANNULA IMPLANTATION PROCEDURE, GENERAL ANESTHESIA IS SUPPLEMENTED WITH PENTOBARBITAL (6.5 MG) VIA THE ARTERIAL CANNULA.

RATIONALE FOR THE IMPLANTATION OF AN ARTERIAL CANNULA ONLY: An arterial cannula is inserted in the right rear leg to measure the MABP. Since the cannulated artery is eventually tied off, no venous cannula is inserted into the right rear leg in order not to compromise the venous return from the same leg. The left rear leg is not cannulated at all because it becomes paretic following injury. Supplemental anesthetic can be safely given through an arterial cannula.

5. An endotracheal tube, smeared with topical anesthetic (2% xylocaine jelly), is inserted after application of local anesthetic (0.5 ml 2 percent xylocaine) to the epiglottis.

6. The cat is mounted in the stereotaxic frame, then

a. the MABP transducer is attached to the arterial cannula;
b. the endotracheal tube is attached to an end-tidal CO_2 monitor;
c. the depth of anesthesia is rechecked using the two criteria described above as well as the MABP and respiratory rates; and
d. the cat may receive supplemental pentobarbital based on the above four criteria as a group. This is a judgment call, as no one criterion is a perfect indicator of the depth of anesthesia. Supplements are given, in aliquots of 6.5 mg, through the arterial cannula.

7. The surgery is performed.

a. An area of the cat's head is shaved, and a 5-cm scalp incision is made.
b. The anterior wall (1 cm x 1 cm) of the right frontal sinus is removed.
c. If the cat shows any signs of discomfort during any of these procedures, supplemental pentobarbital is given as required (6.5 mg, via the arterial cannula).

RATIONALE FOR NOT INSERTING AN ICP PROBE: The ICP probe is not inserted into these cats because

- prior results indicate that the injury caused by a 0.9 joule missile wound causes only a very modest increase in ICP on the average (20 mm/Hg versus 6 mm/Hg for control). This modest rise is not at all life threatening.
- the insertion of the ICP probe could possibly lead to other problems, such as additional brain injury caused by tearing of the ICP probe due to movement of the brain against the stationary probe after the missile injury; thus, insertion of the ICP probe would add nothing to the experiment except the possibility of added nonspecific damage to the brain.

8. ALL cats used in behavioral studies are injured by a 0.9 joule missile ONLY, because there is a greater chance of survival (approximately 70 percent) at this missile energy level.

9. After injury, the scalp incision is sutured. The femoral arterial cannula removed, the artery tied off, and the groin incision sutured. Once assured of adequate respirations, the cat is suctioned through the endotracheal tube; and it is then removed.

10. The cat is given antibiotics (penicillin G, 300,000 U, IM) and topical anesthetic (2% lidocaine jelly) is applied to the sutures (scalp and groin).

11. The cat is returned to the animal care facility and covered with a blanket. AS PART OF OUR STANDARD PROTOCOL, THE VETERINARIAN IS NOTIFIED, AND LACTATED RINGERS SOLUTION (180 cc) IS ADMINISTERED THE NEXT MORNING. ADDITIONAL ANTIBIOTICS ARE ALSO ADMINISTERED BY THE VETERINARIAN FOR THE FIRST 3 DAYS POSTINJURY.

12. Topical anesthetic (2% lidocaine jelly) is applied to the sutures (scalp and groin) once daily for the first 3 days postinjury.

13. The cat is observed daily to determine if it is eating and drinking ad lib. If the cat is not able to eat and drink, the VETERINARIAN IS NOTIFIED and lactated ringer's (180 cc) is given. Most cats are eating and drinking ad lib by the SECOND day postinjury. ALL cats are eating and drinking by day three postinjury.

14. ALL behavioral tests begin on day THREE postinjury and retesting is performed every third day for 30 days, then weekly thereafter for 4 more weeks.

15. NONE of the behavioral tests are traumatic to the cats. The cats are INDUCED to walk the balance beam using canned tuna fish as a reinforcer. Tuna is an excellent reinforcer because NO food deprivation is needed for its reinforcer qualities.

16. NOTE: NO PARALYZING DRUGS ARE GIVEN AT ANY TIME DURING THESE EXPERIMENTS.

Experiments

I. Blood Gas Controls

Initial experiments were concerned with establishing a model for penetrating head wounds. After a decision to use pentobarbital instead of inhalants, the researchers performed experiments to ascertain their ability to control blood gases in anesthetized, paralyzed animals (C1, C2, RC1, RC2). Upon wounding the first animal thusly (M1), the researchers became interested in the effect of wounding on spontaneously breathing animals (M2, M3, M4, M5, M6, M9). Two more paralyzed animals were wounded (M7, M8), but the spontaneously breathing animal was decided to be the more appropriate model. All paralyzed cats were provided with respiratory support as well as additional anesthesia and Pavulon every hour to ensure that they felt no pain. Protocol A was followed with the following exceptions:

- Initial anesthetic induction was achieved via IV injection.
- No ICP transducer was used.
- Cats were sacrificed by rapid decapitation or fix-perfusion.

Updated Information

The purpose of the experiment was to see what anesthesia and wounding did to blood gases. Researchers monitored blood pressure and respirations but not ICP at this time. A straight-on trajectory was used; this was later changed because of the "terrible" brainstem effects observed. The final position of the animal was with the head rotated 20°. [See table III.1.]

Table III.1: Animals Used in the Blood Gas Controls Experiment

Pentobarbital		Pentobarbital with Pavulon	
Control animals	0	**Control animals**	4
Deaths	0	Deaths	0
Study animals	6	**Study animals**	3
Deaths	0	Deaths	0

II. Electrolytes

One of the stated objectives of the first contract proposal was to measure brain water and electrolytes in wounded brain tissue, both immediately and at certain times after wounding. Only eight of these cats were paralyzed and respirated. Protocol A was followed on the acute cats with these exceptions:

- Initial anesthetic induction was achieved via IV injection and cats that required supplementation were given Brevital.
- No ICP transducer was used.
- Cats were sacrificed by rapid decapitation.

The protocol used for the chronic animals was essentially that described in protocol B with these exceptions:

- Initial anesthetic induction was achieved via i.v. injection, and those requiring additional anesthesia were given Brevital.
- Steps 2, 8, 14, 15, and 16 do not apply.
- Cats were sacrificed 1 to 7 days later by rapid decapitation after deep anesthesia was assured.

Updated Information

Seventy-two of these animals were reported on in the Journal of Neurosurgery with the physiology experiment.

The purpose of the experiment was to study brain edema (the accumulation of water in the brain) and determine its occurrence, degree, resolution, and type (that is, vasogenic edema occurs with brain-blood barrier breakdown and cytotoxic edema occurs with lack of blood supply) during and after missile wounds. Determination of edema type is of particular importance. Statistical analysis was done with ANOVA and students. [See tables III.2 and III.3].

Table III.2: Acute Animals Used in the Electrolytes Experiment

Pentobarbital		Pentobarbital with Pavulon	
Control animals	10	**Control animals**	4
Deaths	0	Deaths	0
Study animals	20	**Study animals**	3
Deaths	7	Deaths	0

Table III.3: Chronic Animals Used in the Electrolytes Experiment

Pentobarbital		Pentobarbital with Pavulon	
Control animals	8	**Control animals**	10
Deaths	0	Deaths	2
Study animals	31	**Study animals**	14
Deaths	4	Deaths	4
Rod injury	4	**Rod injury**	6
Deaths	0	Deaths	2

III. Preinjected Evan's Blue Dye

The first contract proposal indicated that the researchers would try to ascertain the effect of the lesion on BBB. To this end, anesthetized cats were injected with Evan's Blue Dye (2%, 2.5 cc/kg) approximately 30 minutes before missile wounding for qualitative determination of BBB breakdown after wounding. For acute cats, protocol A was used with the following exceptions:

- Cats used in earlier experiments were anesthetized initially via IV injection. One cat (M50) was supplemented with Brevital, all others were supplemented with pentobarbital, as needed.
- No ICP transducer was used.
- No paralytic agents were used at any time.
- Cats were sacrificed by fix-perfusion.

For chronic cats, protocol B was used with the following exceptions:

- Initial anesthetic induction was achieved via IV injection.
- Steps 2, 8, 14, and 15 do not apply.
- Cats were sacrificed by fix-perfusion, after deep anesthetic induction, 1 to 3 days after wounding.

Updated Information

In this qualitative study of missile wounding and BBB breakdown and restoration, the hypotheses were that

- missile wounding causes a breakdown in BBB not only around the wound track, but also at a distance from the missile track (that is, causes distant effects);
- missiles of higher energy cause greater disturbances;
- restoration of BBB occurs after missile wounding; and
- missiles of higher energy cause more prolonged damage to BBB (that is, restoration of BBB damage is delayed with missiles of higher energy).

Evan's Blue Dye is probably the most commonly used substance to study gross (qualitative) changes in BBB. The dye has a molecular weight of 67,000. Under the circumstances of this experiment, the dye was found to be poorly tolerated by the animals if given before wounding. There had been no indication prior to using the dye that it would be fatal to the animals.

A rod-induced injury was performed so as to compare a low-energy wound with a high-energy wound caused by the steel sphere. To induce the low-energy wound, researchers used a trephined hole, inserted the rod into the cortex very slowly and pulled it back out. [See table III.4.]

Table III.4: Animals Used in the Preinjected Evan's Blue Dye Experiment

Pentobarbital			
Acute animals		**Chronic animals**	
Control animals	0	**Control animals**	0
Deaths	0	Deaths	0
Study animals	38	**Study animals**	9
Deaths	11	Deaths	6
Failures	5	Failures	0
Rod injury	1	**Rod injury**	1
Deaths	0	Deaths	0

IV. Prostaglandins

These experiments were undertaken to ascertain the effect of the lesion on brain and CSF prostaglandins. For acute cats, Protocol A was used with the following exceptions:

- Initial anesthetic induction was achieved via iv injection.
- No ICP transducer was used.
- No paralytic agents were used at any time.
- Cats were sacrificed by rapid decapitation.

For chronic (24-hour) cats, protocol B was followed with these exceptions:

- Initial anesthetic induction was achieved via iv injection.
- Steps 2, 8, 14, and 15 do not apply.
- All cats were sacrificed the next day by rapid decapitation after deep anesthetic induction.

Updated Information

Prostaglandins are very active substances that (1) may cause vasoconstriction or vasodilatation and (2) may affect nerve function. They are formed from the action of free radicals, which in turn are generated by brain damage; theoretically, prostaglandins can cause secondary brain damage. This damage may be avoided if the generation of biologically active molecules as prostaglandins can be prevented.

The LSU team measured increases in four different CSF prostaglandins. All samples were taken at the times of sacrifice: 5 minutes, 1 hour, and 24 hours after wounding. ANOVA was used for data analysis. Enormous increases were found in the CSF prostaglandins.

All of the acute animals were usable as were all of the chronic animals that did not die overnight. The chronic animals were kept in the animal care facility overnight and returned to the laboratory the next day and sacrificed. [See table III.5.]

Table III.5: Animals Used in the Prostaglandin Experiment

Pentobarbital			
Acute animals		**Chronic animals**	
Control animals	7	**Control animals**	2
Deaths	0	Deaths	0
Study animals	24	**Study animals**	22
Deaths	0	Deaths	5

V. Evan's Blue Dye Injected Postwounding

Because the researchers had difficulty in getting cats pre-injected with Evan's Blue Dye to survive overnight, they decided to inject the dye on the date that animals were to be wounded. These cats were wounded on one day using Protocol B with the following modifications:

- Early animals were anesthetized with pentobarbital (IV).
- Steps 2, 8, 14, and 15 do not apply.
- Cats were sacrificed by fix-perfusion after deep anesthesia and injection of the dye.

Updated Information

Evan's Blue Dye injected the day before wounding was very toxic to the cats. Therefore, in this experiment they were injected with Evan's Blue Dye post wounding. [See table III.6.] The unwounded side of the brain was used as the control. (Note: the animals were shot at 1.0 joules.)

Table III.6: Animals Used in the Evan's Blue Dye Injected Postwounding Experiment

All chronic animals (pentobarbital)	
Control animals	1
Deaths	0
Study animals	44
Deaths	18
Failures	6

VI. Physiology

These experiments were undertaken to ascertain the effects of brain missile wounding on various physiological parameters. These included blood pressure, ICP, blood glucose, hematocrit, blood gases, and end-tidal

CO_2. Three animals (M245, M246, M247) were splenectomized to ascertain whether the observed rise in hematocrit was due to the emptying of the spleen. Protocol A was used for all of these animals with the following modifications:

- All cats were monitored for 6 hours after wounding but were removed from the stereotaxic frame within minutes of wounding.
- Cats were sacrificed by fix-perfusion approximately 6 hours after wounding.

Updated Information

All acute animals were administered pentobarbital, were not paralyzed or ventilated, and received no fluids during the procedure or monitoring period. Blood pressure tracings were recorded and used to manually count heart rate. Heart beats were counted for 10 to 15 seconds and multiplied by 6 or 4, respectively. The end-tidal CO_2 and respiratory rate were monitored. BP-ICP=CPP.

Animals C261, C262, C263, C267, C268, and C269 were controls for all physiology reports. Two animals were wounded at each energy level of 0.9 joules, 1.4 joules, and 2.4 joules.

Analysis of variance was performed using Student Newman-Keuls and ANOVA. Results were considered significant at ± 0.05. [See table III.7.]

Table III.7: Animals Used in the Physiology Experiment

All acute animals (pentobarbital)	
Control animals	8
Deaths	1
Study animals	29
Deaths	6
Splenectomy	3

VII. Coagulation Factors

These experiments were undertaken to ascertain the effects of brain missile wounding on various blood components (platelets) and coagulation factors. Protocol A was used for all cats with the following modifications:

- No ICP transducer was used.
- Cats were sacrificed by either fix-perfusion or an overdose of pentobarbital.

Updated Information

This experiment was not in the scope of the contract, but was an attempt to better define the model. Brain injury may cause abnormality in the blood-clotting system. Although periodically reported in the literature, the principal investigator had not considered this a major clinical problem based on his war neurosurgery experience of over 300 craniotomies. However, at LSU, some animals were developing blood clots in their brains; the researchers wanted to determine clotting times.

They found evidence of platelet clumping and clotting-factor component changes within minutes of wounding. Despite these laboratory changes, the animals exhibited no unusual clinical problems, such as excessive scalp bleeding or bleeding from the groin incision. [See table III.8.]

A paper was prepared on the experiment, but was not accepted by the Journal of Neurosurgery.

Table III.8: Animals Used in the Coagulation Factors Experiment

All acute animals (pentobarbital)	
Control animals	5
Deaths	0
Study animals	8
Deaths	0

VIII. Histology

To obtain good histological records of missile-wounded brains, the researchers performed experiments which were terminated at time points corresponding to the sacrifice times of earlier experiments (i.e., 1 minute, 5 minutes, 1 hour, etc.). Protocol A was used for all acute cats, which were sacrificed by fix-perfusion. Protocol B was used for all chronic cats with the following modifications:

- Steps 2, 8, 14, and 15 do not apply.
- All cats were sacrificed by fix-perfusion after deep anesthetic induction.

Updated Information

The purpose of this experiment was to look at tissue changes in dead brain cells. For an effective use of resources, this experiment relied, in part, on animals which were scheduled for but not used in other experiments. This experiment was performed during 1 year. [See table III.9.]

Table III.9: Animals Used in the Histology Experiment

Pentobarbital			
Acute animals		**Chronic animals**	
Control animals	1	**Control animals**	0
Deaths	0	Deaths	0
Study animals	18	**Study animals**	6
Deaths	0	Deaths	1

IX. Behavior

Three groups of cats were used for behavioral assessment. The first two groups established and validated behavioral and reflex tests that could be used to discriminate injured from noninjured cats. The third group represented actual experiments testing injured, non-drug-treated cats versus injured, GM1-ganglioside treated cats. Protocol B was used for all groups. Groups 1 and 2 included some cats anesthetized with isoflurane. Step 2 of Protocol B does not apply to the first two groups.

Updated Information

The principal investigator did not initially propose chronic studies because he is not a pharmacologist. One of the researchers was responsible for this change in the direction of the chronic studies; however, different researchers tested the three groups of animals used in this experiment. The researchers found it very difficult to use ordinary animals for their behavior studies because they are "fierce" and very difficult to handle. A "breeding program" was thus instituted to obtain "reasonable" animals, which allows animals that arrive pregnant at LSU to deliver before being used for experimentation.

GM1-ganglioside was selected because, at the time, there was evidence it was effective for small, selective lesions in rats, and it had been used for human fetal transplants of tissue in the brain.

Under pentobarbital, the animals were sleepy for 1 to 2 days; this permitted a 3-day window in which to measure the effects of any test drug. Researchers searched extensively for short-acting anesthetics so animals would wake up immediately after wounding and allow for more days to test the drug effects. As a result, different anesthetics were used: 13 animals were given isoflurane. [See table III.10.]

Table III.10: Animals Used in the Behavior Experiment

	Group 1		
Pentobarbital		**Isoflurane**	
Control animals	0	**Control animals**	0
Deaths	0	Deaths	0
Study animals	3	**Study animals**	6
Deaths	1	Deaths	1
	Group 2		
Pentobarbital		**Isoflurane**	
Control animals	0	**Control animals**	1
Deaths	0	Deaths	0
Study animals	10	**Study animals**	6
Deaths	5	Deaths	5
	Group 3		
Pentobarbital			
Control animals	0		
Deaths	0		
Study animals	27		
Deaths	12		

X. Cerebral Blood Flow

Preliminary experiments to determine changes in regional cerebral blood flow in missile wounded brains were performed by a neurosurgical resident. Protocol A was used with the following modifications:

- Cannulations included one femoral vein, both femoral arteries, and both brachial arteries.
- After achieving adequate anesthesia, all cats were paralyzed with gallamine triethiodide and placed on a respirator. Proper anesthesia and paralyzation were maintained.
- All cats were sacrificed by fix-perfusion.

Updated Information

This work was performed as part of the second contract. These experiments were either 2 hours or less (acute) in duration. The first measurement was taken about 30 minutes before wounding, and the last was taken about 90 minutes after wounding. The gallamine dose was 10 to 15 mg/kg to paralyze the animals; usually, 30 mg of gallamine was administered as it was longer-acting and required no supplementation. Some animals were ventilated with room air supplemented with O_2. ANOVA and Tukey's statistical programs were used from SASS. [See table III.11.]

Table III.11: Animals Used in the Cerebral Blood Flow Experiment

All acute animals (pentobarbital with gallamine)	
Control animals	16
Deaths	3
Study animals	29
Deaths	1

XI. Apnea

These experiments were performed to tighten and complete earlier data compiled on the apneic response. Protocol A was used on all cats. All cats were sacrificed either by fix-perfusion or barbiturate overdose.

Updated Information

Initial data on apnea was developed on animals that were used to measure electrolytes or those that were wounded but not paralyzed. Additional animals were used in order to better define the issue. When an injured animal became apneic, the researchers ventilated the animal if it did not breathe on its own after about 1 minute. Animals designated as apneic resumed breathing on their own within 6 minutes. They were designated as dead if they did not begin breathing on their own within 6 minutes, while life was maintained with respiratory support. This experiment was reported in the Journal of Neurosurgery article. [See table III.12.]

Table III.12: Animals Used in the Apnea Experiment

All acute animals (pentobarbital)	
Control animals	1
Deaths	0
Study animals	16
Deaths	2

XII. Plasma Catecholamines

These experiments were performed to determine (1) the time course of the sympatho-adrenal response as reflected by plasma levels of the catecholamines norepinephrine and (2) if the plasma catecholamines response followed a similar time course when ICP was increased WITHOUT injury. Additional experiments were also performed to determine if the angle of the trajectory had any effect on the time course of the plasma catecholamine response. Protocol A was followed for all cats. Cats were sacrificed by rapid decapitation using a large animal decapitator.

Updated Information

Plasma catecholamines are an indication of physical or psychological stress. When their level is high, it indicates a potent fight or flight response. With the breakdown in BBB, these potent factors may enter the brain and affect its response. Normally, however, they do not enter the brain. Low-energy wounds at 0.9 joules were much less stressful than high-energy wounds. Researchers also measured plasma glucose (released by epinephrine), which is another stress indicator.

All of these experiments were acute, lasting only 60 minutes. Plasma samples were taken at different intervals out to 60 minutes.

The researchers inserted a spinal needle into the cisterna magna at the base of the skull. The needle was connected to a fluid column of mock CSF. Lowering and elevating the mock fluid in the bottle changes the ICP in the animal's head. This was performed over the same time course as with the catecholamine animals, for control; that is, to determine whether the injury was attributable only to, or mostly to, increased ICP. The researchers tried to mimic the rapidity of the elevated ICP resulting from the missile wound. They concluded that the injury is distinct from increased ICP. [See table III.13.]

Table III.13: Animals Used in the Plasma Catecholamines Experiment

All acute animals (pentobarbital)	
Control animals	0
Deaths	0
Study animals	22
Deaths	5
Fluid column	10
Transverse injury	4

XIII. Brain Catecholamines

The biogenic amines serotonin, norepinephrine, epinephrine, dopamine and their metabolites were measured in 15 different brain areas immediately after injury at three different velocity levels to determine if these velocity levels affected the biogenic amine levels differently in the brain areas selected. Protocol A was used for these experiments. Cats were sacrificed by rapid decapitation.

Updated Information

This exploratory work was almost complete as of June 1989. If an effect was found with a 2.4 joules injury, tests were to have been done at lower energies as well. If not, work was to have stopped in this area. Three groups were involved: group 1 was control, group 2 was ICP control, and group 3 was animals wounded at 2.4 joules. Measurements were taken at 6 minutes postwounding in order to evaluate the immediate effect on the cardiac, respiratory, and hypothalamic areas of the brain. They found that the hypothalamic area was immediately effected. [See table III.14.]

Table III.14: Animals Used in the Brain Catecholamines Experiment

All acute animals (pentobarbital)	
Control animals	0
Deaths	0
Study animals	27
Deaths	1

XIV. Recovery

These animals were the first to be allowed to recover from anesthesia and were observed for behavioral deficit. M23A subsequently died about 2 years later from unrelated causes, while M24 is still housed at LSU and appears to be quite normal. The protocol used was essentially that described in protocol B with the following modifications:

- Both cats were anesthetized via iv injection, M23A was given Brevital and M24 was given pentobarbital.
- Steps 2, 8, 14, and 15 do not apply.

Updated Information

M24 is still at LSU; the animal was wounded at 1.4 joules. (See table III.15.)

Table III.15: Animals Used in the Recovery Experiment

Anesthesia	Animal
Brevital	1
Pentobarbital	1

XV. Glucose Catecholamines

This experiment was an early attempt to collect data on plasma glucose and catecholamines. The technique and assay were not worked out until later. Protocol A was used with the following exceptions:

- Anesthesia was induced with pentobarbital (iv)
- Cat was given Pavulon and placed on a respirator.
- Cat was sacrificed by fix-perfusion.

Updated Information

This experiment was suspended because the laboratory did not have its own HPLC. [See table III.16.]

Table III.16: Animals Used in the Glucose Catecholamines Experiment

Anesthesia	Animal
Pentobarbital with Pavulon	1

XVI. Photog

This experiment was an attempt to understand inhalant anesthesia, which proved to be very tricky in cats. Protocol A was followed with these exceptions:

- Anesthesia was achieved with 3 percent halothane and maintained with nitrous oxide and oxygen.
- Cat was paralyzed with Pavulon and placed on the respirator.
- Cat was sacrificed by fix-perfusion. [See table III.17.]

Table III.17: Animals Used in the Photog Experiment

Anesthesia	Animal
Halothane and nitrous oxide and Pavulon	1

XVII. Blood Contamination

These experiments were performed to determine to what extent blood contamination in brain tissue changes the obtained percent of water and electrolyte determinations. Protocol A was used with the following exceptions:

- Pentobarbital was injected (iv).
- Cat was sacrificed by rapid decapitation.

Updated Information

In this experiment the same amount of brain and solvent were dissolved with different amounts of blood to evaluate its effects spectrometrically. Because the data results did not indicate a linear curve, work was suspended. [See table III.18.]

Table III.18: Animals Used in the Blood Contamination Experiment

Anesthesia	Animals
Pentobarbital	2

XVIII. Audio-Evoked Potentials

These experiments were performed with the aid of Charles I. Berlin, Ph.D., of the Kresge Hearing Laboratory. The purpose was to determine the effects of brain missile wounding on evoked auditory response. Protocol A was essentially followed with these exceptions:

- Pentobarbital anesthesia was induced iv.
- No ICP transducer was used.
- Cats were sacrificed either by fix-perfusion or barbiturate overdose.

Updated Information

These experiments were conducted to determine the effect of the missile on the brainstem. One method of studying this effect is to trace electricity through the brainstem by exposing the ear to sound (which is then transmitted through the eighth nerve to the cortex) and measure the integrity of the pathway. These experiments were also an unsuccessful attempt to collaborate with other researchers. [See table III.19.]

Table III.19: Animals Used in the Audio-Evoked Potentials Experiment

Anesthesia	Animals
Pentobarbital	3

XIX. Pulmonary Edema

These experiments were performed during a period when the researchers were losing many cats to respiratory problems. The main goal was to determine whether these cats were developing pulmonary edema. Protocol A was used with the following modifications:

- Pentobarbital was administered iv.
- No ICP transducer was used.
- Cats were sacrificed by rapid decapitation.

Updated Information

Many animals were dying of respiratory failure. This experiment was to determine if the missile wound was causing pulmonary edema or fluid to accumulate in the lung. The wet lung was weighed, dried, and weighed again to determine fluid content. This work was continued in groups 8 and 9 (see pp. 209-210). [See table III.20.]

Table III.20: Animals Used in the Pulmonary Edema Experiment

Anesthesia	Animals
Pentobarbital	4

XX. TTC-BBB Breakdowns

These experiments were performed using a different substance to demark areas of BBB breakdown. However, the chemical chosen did not prove to be as reliable as Evan's Blue Dye. Two of these experiments were performed using Protocol A and were terminated by fix-perfusion. The third was performed using Protocol B with the following exceptions:

- Steps 2, 8, 14, and 15 do not apply.
- Cat was sacrificed the next day by fix-perfusion after deep anesthesia was achieved.

Updated Information

TTC is a chemical used to measure brain metabolism, which can be used as an index of brain blood flow. TTC is injected IV; it proved to be difficult to control. On the basis of this work, the principal investigator did not find ischemia a problem. [See table III.21.]

Table III.21: Animals Used in the TTC - BBB Breakdowns Experiment

Anesthesia	Animals
Pentobarbital	3

XXI. Circling (Isoflurane)

To determine whether the observed circling behavior in wounded cats was due to a field cut, two cats were operated on so that the optic cortex was oblated. Protocol B was used with the following modifications:

- Surgery was performed under inhalant anesthesia (isoflurane).
- Steps 2, 8, 14, and 15 do not apply.
- Several days later, cats were reanesthetized and sacrificed by fix-perfusion.

Updated Information

This experiment was to determine if this circling was caused by impaired vision. If the animal was wounded, the missile would damage the optic cortex or part of the animal's brain that controls vision. To test this hypothesis, the researchers trephined out a small hole in the skull and suctioned out a portion of the optic cortex in the animal's brain. The animals were then allowed to recover. The researchers observed that these animals did not walk in circles as those animals allowed to survive after wounding. (See table III.22.)

Table III.22: Animals Used in the Circling Experiment

Anesthesia	Animals
Isoflurane	2

XXII. Left Ventricular Cannula

These experiments were performed to attempt to perfect a cerebral ventricular cannula for chronic measurement of ICP and CSF sampling. Protocol B was used with the following changes:

- Steps 2, 8, 14, and 15 do not apply.

- Cats were later reanesthetized and fix-perfused.

Updated Information

The purpose of these experiments was to conduct long-term ICP measurements and CSF sampling. The researchers planned to use this technique on the behavior study animals, which survived 90 days after wounding. The researchers found that they could manage this cannula, but decided against using it because it traumatized the animals further. [See table III.23.]

Table III.23: Animals Used in the Left Ventricular Cannula Experiment

Anesthesia	Animals
Pentobarbital	4

XXIII. Anesthesia

This experiment represented the beginning of a series performed to determine the effects of different anesthetic agents on the apneic response. Protocol A was used with the following changes:

- Isoflurane anesthesia was used.
- Cat was sacrificed by fix-perfusion. [See table III.24.]

Table III.24: Animals Used in the Anesthesia Experiment

Anesthesia	Animals
Isoflurane	1

XXIV-XXXIII. Study of Brain and Organ Blood Flow

These experiments were performed by one researcher using the following procedures:

All cats in this project were initially anesthetized with 30-40 mg/kg pentobarbital IP. Smaller supplemental and maintenance doses of pentobarbital were administered IV during the surgical procedures to achieve a stable and relatively deep level of anesthesia for determination of regional cerebral blood flow, organ's blood flow, and other physiological parameters.

The adequacy of anesthesia was first tested by painless mechanical stimuli, such as stretching the arms, legs, or pressing against the paws. If no reflexes (e.g., limb withdrawal) were noticed, the cat was placed supine (on its spine) for femoral venous and arterial cannulation. Additionally, small doses of a local anesthetic were injected under the skin a few minutes before an incision was made. Immediately after a femoral arterial cannula and venous cannula were inserted, the MABP was continuously monitored. Adequacy of the anesthesia was then judged by the stability of MABP and heart rate. Small doses of pentobarbital were administered at appropriate times as listed for individual cats.

Invasive surgical procedures were started only following a relatively deep and stable period of anesthesia. Upon completion of surgery, cats were paralyzed by an IV injection of 30-40 mg gallamine, except for groups 8 and 9. No anesthetic or paralytic materials were given during a 100 to 120 minute period of experimentation. At this period however, cats had a stable and deep anesthesia as judged by either MABP, heart rate, or EEG [electroencephalography] recordings—whichever was applicable under the specific experimental conditions.

After the experimental period which usually lasted 100 minutes and in no cases over 120 minutes, cats were euthanized by a lethal dose of pentobarbital IV. At this final stage, usually 30 to 40 mg of pentobarbital produced a sharp fall in MABP followed immediately by a flat EEG (brain death), indicating that cats must have been deeply anesthetized during the preceding experimentation period.

Group 1: Controls Unwounded (NU)

Purpose: To study (1) autoregulation and pattern of regional blood flow in the brain and (2) redistribution of cardiac output in the heart, kidney, spleen, spinal cord, adrenals, and muscle during a 100-minute period of experimentations planned for groups 2 and 4. [See table III.25.]

Table III.25: Animals Used in Experimental Group 1

Control animals	8
Incomplete	1

Group 2: Wounded or Normotensive (1.4j NI)

Purpose: To study (1) autoregulation and pattern of regional blood flow in the brain and (2) redistribution of cardiac output in the heart, kidney, spleen, spinal cord, adrenals, and muscle following brain missile wounding. [See table III.26.]

Table III.26: Animals Used in Experimental Group 2

Study animals	9
Incomplete	2

Group 3: Unwounded Hypotensive (HU)

Purpose: To study (1) autoregulation and pattern of regional blood flow in the brain and (2) redistribution of cardiac output in the heart, kidney, spleen, spinal cord, adrenals, and muscle during graded hypotension and after blood reinfusion. [See table III.27.]

Table III.27: Animals Used in Experimental Group 3

Control animals	11
Incomplete	4

Group 4: Wounded Hypotensive (1.4j HI)

Purpose: To study (1) autoregulation and pattern of regional blood flow in the brain and (2) redistribution of cardiac output in the heart, kidney, spleen, spinal cord, adrenals, and muscle following brain missile wounding associated with graded hemorrhagic hypotension and after blood reinfusion. [See table III.28.]

Table III.28: Animals Used in Experimental Group 4

Study animals	14
Incomplete	4

Group 5: Hypercapnia (HC)

Purpose: To study the reactivity of regional cerebral blood flow to high arterial CO_2 (hypercapnia) before and after brain missile wounding. [See table III.29.]

Table III.29: Animals Used in Experimental Group 5

Study animals	8
Incomplete	1
Partially complete	2

Group 6: Hypoxia (LO)

Purpose: To study the reactivity of regional cerebral blood flow to low arterial O_2 (hypoxia) before and after brain missile wounding (10 percent O_2). [See table III.30.]

Table III.30: Animals Used in Experimental Group 6

Study animals	7
Partially complete	3

Group 7: Hyperoxia (HO)

Purpose: To study the reactivity of regional cerebral blood flow to high arterial O_2 (hyperoxia) before and after brain missile wounding (100% O_2).

Updated Information

The study question was "Is giving additional O_2 good or bad?" It is thought that giving additional O_2 will lower blood flow to the brain. The researchers tested the chemical regulation by giving too much O_2. The findings were (1) O_2 does not affect all areas of the brain the same way at the same time in the animal, (2) brain missile wound enhances vasoconstriction in the brain, and (3) additional O_2 further restricts blood flow in some areas of the brain. [See table III.31.]

Table III.31: Animals Used in Experimental Group 7

Study animals	10
Incomplete	1
Partially complete	3

Group 8: Respiratory Unwounded (RU)

Purpose: To control unwounded cats for cerebral, cardiovascular, and respiratory effects of brain missile wounding in spontaneously breathing cats. [See table III.32.]

Table III.32: Animals Used in Experimental Group 8

Control animals	6
Incomplete	1

Group 9: Respiratory Injured (RI)

Purpose: To study brain missile-wounded cats for cerebral, cardiovascular, and respiratory effects of wounding in spontaneously breathing surviving and nonsurviving cats.

Updated Information

RU and RI animals were not ventilated, but were spontaneously breathing with no respiratory support. The purpose was to answer the question "What are the differences between survivors and non-survivors?" Blood flow was not interrupted to the brain stem.

An additional purpose of groups 8 and 9 was to prove that the brain loses control of the body's organs. These organs then function at their own rates and can cause a secondary insult to the brain, for example, through the heart's control of blood flow and pressure, the lungs' control of O_2, and the kidneys' control of toxins. The conclusion is that if the organs are supported and controlled until the brain can heal, the animal death rate could be cut substantially. [See table III.33.]

Table III.33: Animals Used in Experimental Group 9

Study animals	21
Incomplete	8

Group 10: Hypocapnia-Hyperventilation (LC)

Purpose: To study the regional cerebral blood flow responsiveness to low blood CO_2 before and after brain missile wounding.

Updated Information

The researchers hypothesized that following brain missile wounding cerebral vasoconstriction from hypocapnia may further reduce cerebral blood flow. An additional decrease in regional cerebral blood flow may thus counteract any beneficial effect of hyperventilation in reducing ICP. Regional cerebral blood flow was significantly reduced in 7 of 14 brain structures before brain missile wounding in 5 animals. [See table III.34.]

Table III.34: Animals Used in Experimental Group 10

Study animals	14
Incomplete	5
Partially complete	4

Group 11: Hyperoxia Hypercapnia: CBF Reactivity Before and After Brain Missile Wound (HH)

Table III.35: Animals Used in Experimental Group 11

Study animals	3
Incomplete	0

Group 12: Survival and Neurological Deficiencies in Apneic Animals (Apnea)

Table III.36: Animals Used in Experimental Group 12

Study animals	4
Incomplete	1

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Comments From Individual Members of GAO's Medical Panel

On June 19, 1989, GAO's medical panel met to discuss aspects of the research: goals, methodology, and value; experimental model and animal care; anesthetic controls; and investigator qualifications and equipment. The discussions in each of these areas focused on both the research as proposed and the research as performed. Discussion centered on a series of questions we developed before the meeting. At the end of the discussion on each section, each panelist wrote his or her responses to the questions in a workbook. At the end of the meeting, we collected the workbooks from each panelist and sent copies to the chairman, Dr. John A. Jane, who summarized the responses. (See app. VI.)

This appendix provides all of the written comments of each panelist, which we have edited slightly for correct punctuation, syntax, and abbreviation use. The panelists are identified by numbers, 1 through 8, that were randomly assigned. The name of the panelist has not been associated with his or her comments, but all comments attributed to a panelist were made by the same panelist.

I. Research Goals/ Methods and Value

A. Research as Proposed

First Question: Was the proposed research based on hypotheses that were medically valid at the time?

[1] Yes! The hypotheses are broadly stated and do not fall into a traditional NIH [National Institutes of Health] format; however, they do appear consistent with the state-of-the-art of this particular field!

[2] Yes. GSW [gunshot wounds] are important for military and civil warfare. A model with a dose-response curve was needed to test hypothesis of treatment. The investigators have done well to develop such a model—even if it has not been reviewed and published. The use of a cat is very well justified by the investigators. The hypotheses proposed in the second proposal are appropriate, although they could be better stated.

[3] Yes. Important topic with major limitations in our knowledge. Planned to (1) develop model, (2) enhance understanding, (3) modulate response. Worthwhile and valid approach.

[4] This is an interesting proposal dealing with the important area of head injury, via missile head wounds. While the hypotheses are broad, that is, broadly stated, they are important and clear: to develop a head injury model which is systematic and reproducible, in which a variety of therapeutic and pharmacologic interventions can be tested. This is of importance and the PI [principal investigator] has done this well—i.e., his approach is valid.

[5] In a word, yes. (1) The question of whether different approaches to therapy of penetrating head wounds would help recovery of human subjects was certainly open. (2) A model for testing therapeutic manipulations was needed. (3) The proposal outlined development of a model to test the effect of drugs on survival and morbidity.

[6] Yes, I feel (although written in a generic blanket way) that the hypothesis was both medically valid and important. The PI has developed an important animal model for application of missile injury for both DOD and the civilian community.

[7] Hypotheses were very broad. They had previously been looked at in a primate model (Crockard, et al.). To look at area of missile tract vs. brainstem injury is valid.

[8] [No comment]

Second Question: Did the proposed research have value considering the body of knowledge on the treatment of brain wounds at the time?

[1] Yes! The choice of all therapeutic strategies appears [to be] based on contemporary thought.

[2] Yes. Treatments selected were in line with contemporary thinking on mechanisms of damage to the CNS [central nervous system].

[3] Yes. Anticipated using most potentially useful drugs. Perhaps barbiturate needs to be added.

Question outcomes discrete enough to measure some of effects.

[4] Yes, without doubt. It was valid when it was first proposed and is so now as well. We knew (and know) little concerning the treatment of gunshot wounds, both in military situations and daily life in our cities; this study could help in this regard.

[5] [Respondent referenced his or her previous answer.] In a word, yes. (1) The question of whether different approaches to therapy of penetrating head wounds would help recovery of human subjects was certainly open. (2) A model for testing therapeutic manipulations was needed. (3) The proposal outlined development of a model to test the effect of drugs on survival and morbidity.

(1)—The best or most effective therapy for reducing morbidity and mortality after a penetrating head wound was not and still is not established. A model and an approach for evaluation was then and still is needed.

[6] Yes, the PI has developed a working model for which all contemporary treatment (drug) strategies can be tested. The panel reinforced the need for "newer knowledge" about treating fragmented missile injuries.

[7] Yes, although endpoints are weak. While "drug testing" proposal was valid, to date they have not been carried out!

[8] [No comment]

Third Question: Did the proposed research attempt to provide information on so many variables that it would be difficult to address any one of them thoroughly within proposed time and resources?

[1] No! The variables are broad-based and may, upon superficial examination, appear diffuse. Yet, again, the nature of the questions asked mandate such an approach. Importantly, the variables assessed show focusing as the application progresses.

[2] In view of the paucity of information available, it was appropriate to look at this large group of variables (CBF [Cerebral Blood Flow], CMROS, ICP, etc.). Statistical analysis, however, is not discussed to indicate how these variables would be handled. Anything less would probably not have been approved for funding.

[3] It was necessary to collect all data to describe the model.

Physiological and biochemical response to discrete injury problems. Staff [went] from physiologic focus to biochemical focus warranted (proposal 1-2).

[4] No. In this type of descriptive proposal, variables must be measured. He [the PI] has lots of variables, but they are probably all of importance. To correlate the physiological, metabolic, pathologic, and neurological functional variables is important. If he had not said he would make these measurements, we would criticize him for not doing so. I would like to have seen precisely how all these correlations would be accomplished. This was somewhat deficient in the proposal.

[5] The original proposal was exploratory and listed a large number of variables to be evaluated. It certainly outlined an optimistic view of probable accomplishments.

[6] As with any good proposal, there are a large number of important variables. The PI emphasized the ones he thought were more important at the time of the proposal. As the experiment progressed, he gained the knowledge of both the experimental data and what new knowledge has progressed in the literature (and reemphasized new variables). It would have been clearer had the PI provided the statistical relationship between these variables.

It could be helpful to know if the Army viewed this "contract" as a deliverable contract or as a "grant" with flexibility.

[7] Absolutely. Also areas of investigation were entered into that were not part of the original design, namely the biochemical measurements. These were not part of the first proposal but were investigated during the first time period, even though the areas which were to be investigated, i.e., drug treatment, still have not been looked at!

[8] [No comment]

Fourth Question: Was the proposed research taking an overly simplistic view of a more complex problem?

[1] The view is "simplistic"; however, when little is known about the sequelae of the injury, such a simplified approach is valid.

[2] Simplified, yes, but not "simplistic."

[3] Reasonable trade-offs were made in quest for a reliable model.

[4] This issue is a very complex one, which the PI probably understated in the proposals. I think he attempted to come up with the simplest model possible.

[5] The original proposal did simplify a problem that is inherently complex. It proposed a realistic model and a series of approaches. The model was more difficult to evolve than anticipated. In this, the original proposal (1983) did not anticipate all of the difficulties (ballistic device problems, restriction on experiments, lesion variables).

[6] In spite of the many variables (and in hindsight) I feel the PI oversimplified the problem. His approach of going with a sphere rather than a multishaped fragment—I think it was done appropriately. It at least offered a model of reproducibility and predictability.

[7] Yes.

[8] [No comment]

Fifth Question (a): Did the proposals clearly and specifically indicate, for each type of experiment, a methodological design for interpreting/analyzing the data?

[1] This is a weakness in this study. Overall detail is lacking! Problems and pitfalls are not addressed, and a precise plan of data analysis is not given.

[2] I feel this is an area of weakness in the proposals.

[3] No.

[4] This is one area which was deficient. How the correlational analyses were to be done [is] unclear; which statistical analyses are done are not clear. Incomplete statistical analysis.

[5] No.

[6] No, not clearly and specifically. The PI proposed and executed a series of experiments for the proposal. The proposals could have been written a little tighter, especially the second proposal. In essence, the

proposals were overall research objectives, not specific protocols designed to fit in the pieces of the puzzle.

[7] No.

[8] [No comment]

Fifth Question (b): Did the proposals clearly and specifically indicate, for each type of experiment, a step-by-step description of the research methods and procedures to be used?

[1] Descriptions are rather brief and detract from the proposal. Perhaps page limitations contributed to this.

[2] As well as can be expected in an unknown area.

[3] No—methods and experiments changed.

[4] Some of the procedures and methodologies are not well described. His techniques are not completely described. Is this because of page limitations?

[5] [No comment]

[6] Not, specifically, although logically I find it acceptable. It would have been helpful to have a progress report on the proposals with acquired data. An overall research plan with specific supporting protocols would have been desirable.

[7] Yes, but they were not followed in some instances.

[8] [No comment]

Fifth Question (c): Did the proposals clearly and specifically indicate, for each type of experiment, the number of animals needed to achieve statistically significant results?

[1] I cannot answer this clearly! The plan of statistical analysis is not clearly presented. Perhaps, with a high failure rate, more animals may be required.

[2] This issue was not addressed—I cannot determine how the "N" of any group was arrived at.

[3] No power analyses? Would have contributed. Generally stated that numbers are limited based on tight results probably appropriate for proposal as carried out.

[4] He states that he will utilize 200 animals/year; i.e., 4/week. This is probably not excessive for the many proposals the PI has outlined, and considering the fact that three investigators are working on this project.

[5] Hard to predict in advance.

[6] No. I was impressed with the low numbers of animals used, however, for each study. I question if he used sufficient numbers of animals (n=5) to draw conclusions. Without an overall statistical plan, including experimental failures, it's tough to speculate. Detailed data analysis planning should have been given.

[7] No! Statistical analysis was not the strong point of this protocol.

[8] [No comment]

Sixth Question (a): Was there evidence in the proposals that a thorough literature search had been conducted for all work (published and unpublished) for each type of experiment?

[1] Not extensive, yet adequate for the application.

[2] Adequate and appropriate for the purposes of the proposed study.

[3] No. Generally well covered—some areas better than others.

[4] The PI, I believe, covered the literature areas related to the proposal. He missed a few, but most are not important to the proposal.

[5] Certainly not, but the question is unreasonable. He covered the available literature on head wounds reasonably well, but did not discuss ballistics and missile shape versus tissue injury.

[6] No, is that possible? I think he did a fairly good job on current published work relating to this effort.

[7] No.

[8] [No comment]

Sixth Question (b): Was there evidence in the proposals that a thorough literature search had been conducted for all work in forensics, military science, law enforcement, and recreational firearms pertaining to the shape, size, weight, and velocity of the missiles and characteristics of injuries they cause?

[1] [Respondent referenced his or her previous answer.] Not extensive, yet adequate for the application.

[2] Not relevant for the proposed work.

[3] No. Probably less than adequate. Simplified version—model not easy to relate to circumstances of injuries in war.

[4] No, much of this was not covered, but my view is that much of this is unnecessary for the proposal.

[5] [No comment]

[6] No, but focused on that literature relating to producing a model very well.

[7] No.

[8] [No comment]

Seventh Question: Were the proposed treatment drugs likely to provide information immediately transferable to humans with penetrating head wounds?

[1] Yes, the list of drugs is extensive and the endpoints are soft; however, given contemporary clinical interest, their choice seems appropriate and transferable to humans!

[2] Appropriate in light of contemporary knowledge. Results could be transferred to clinical trials. It would be wrong to suggest "immediate" transfer. This is a fault of the writer of this question, not the investigator!

[3] Information on apnea is transferable. Drug results may or may not be [transferable] when available. Important comments from [Panelist #1] RE: cat model and apnea.

[4] Yes. As far as any drug tested on humans which has been demonstrated to be effective on animals. The PI has chosen the appropriate drugs which may be effective. From a scientific viewpoint, I would have preferred to see a more mechanistic approach with fewer drugs, rather than a "shotgun" approach to all drugs.

[5] If these studies were carried out, they would be valuable additions to treatment options for human injuries. This puts the suggested drugs for the 1983 proposal and the 1985 [Sentence unfinished].

[6] Yes, especially the apnea support in the acute injury.

[7] Yes.

[8] [No comment]

Eighth Question: Is the steel sphere realistic for simulating battlefield fragment wounds? Is scaling of the sphere used to inflict the injury an issue? Can the scaling problem, if any, be handled in data interpretation? If so, how?

[1] Yes, the sphere is not perfect. It does not faithfully replicate the battlefield situation; however, it is the best that can be done to achieve experimental rigor.

[2] Good choice for an experimental model, even though there are many differences between a sphere, a bullet, or shrapnel.

[3] No. Sphere needed for consistent model. [Therefore it is the] best choice. Extrapolation an issue. Re fragment wounds. Good model.

[4] The steel sphere doesn't realistically simulate every condition, but does have some applicability to some bullet wounds here. It's not completely representative, but it's not a major factor.

[5] Realistic for battlefield—no.

Certainly a reasonable approach for development of a model.

Scaling problem was addressed in proposal but not followed through in the analysis presented.

[6] Yes. The injury induced by steel spheres is much more uniform than that with fragments. It does, however, offer a predictable, measurable starting point which could be transferred to comparable modeling. I feel scaling of the sphere is important. The scaling problem may be handled in data interpretation (e.g., correlating analogous missile injury in humans).

[7] No, it does not. It is an issue, but the panel could not come up with any better model.

[8] [No comment]

Ninth Question: Does the method of wounding predetermine a part of the brain that may be resistant or susceptible to injury? Does the trajectory of the missile result in a wound that leaves the animal neurologically intact with the likelihood that full neurological recovery would result without any treatment?

[1] The injury does not leave the animal intact and the likelihood of full neurological recovery seems remote.

[2] Animals have neurol[ogical] deficits and are therefore appropriate subjects for treatment studies.

[3] Animal not intact. Wound appropriate for interventions that are contemplated—given modifications in measures of neurologic deficit.

[4] The method of wounding does indeed determine which part of the brain is injured. His deficit is scarce enough, however, to be able to successfully test the pharmacologic interventions.

[5] The wounding procedure certainly would not and did not leave models neurologically intact. Therefore, the trajectory of the missile chosen was not one that would have left animals intact neurologically if they were properly evaluated (as current progress report suggests).

[6] Yes, especially by varying the missile. This is a difficult energies question. Neurologic insult from a missile fragment will induce some irreversible changes. Changing the trajectory angle did provide a method of inducing injury without jeopardizing the animals' lives.

[7] Wounding takes an A-P [anterior-posterior] direction in the cranium and therefore goes through both gray and white matter—missing the

brainstem. The wound has been "graded" in the sense that different energies have been used.

[8] [No comment]

Tenth Question: Are the data gained from this research limited because the anterior wall of the right frontal sinus is removed prior to injury?

[1] No!!

[2] No.

[3] No—well explained.

[4] It's certainly a bit [of a] different model, but, I do not believe this frontal sinus removal will limit the data gained.

[5] Not given the underlying purpose—The aim was to produce a reproducible model, not to replicate all of the [Sentence unfinished].

[6] No. Data is influenced, but I feel the PI adequately addressed this question by maintaining an intact skull.

[7] No.

[8] [No comment]

Eleventh Question: If you were assessing the research at the time it was proposed, overall, how would you rate its goals, methods, and value?

	5 Very high	4 Somewhat high	3 Neither low nor high	2 Somewhat low	1 Very low
1.			X		
2.			X		
3.			X		
4.		X			
5.		X			
6.		X			
7.			X		
8.	X				

[1] [No comment]

[2] [No comment]

[3] Goals—high; methods—OK; and value—somewhat high to debatable.

[4] [No comment]

[5] [No comment]

[6] [No comment]

[7] [No comment]

[8] [No comment]

B. Research as Performed

First Question: Is the research completed to date based on the proposed hypotheses?

[1] Although many research questions have not been explored, progress has been made, and, overall, such progress seems reasonable!

[2] First proposal—establishing a model has been done. They are behind schedule in the drug evaluation studies. The plan, however, is valid.

[3] Research follows a systematic plan and valid approach. Research is on track.

[4] The work is still incomplete. I believe they are on track, but much more needs to be done. They have developed [a] reliable model, now let's see what they can do with it.

[5] In part only.

A model has been developed and reasonably validated. A variety of measurements, e.g., blood flow, chemical [Sentence unfinished].

[6] No, not as originally proposed. Objectives of protocol were to develop model, study physiologic (missile-induced) alterations, and begin drug testing. PI has not begun the third area to date. Research completion, however, is on an aggressive track. It would be helpful to know if the contract was really a "contract" or was treated as a "grant."

[7] No, no evaluation of drugs. Given long amount of time that has elapsed—human data and especially as pertaining to decoding has come to light. They may want to "re-evaluate" which drugs they want to test in future—if the project continues.

[8] [No comment]

Second Question: Is the research completed to date increasing the body of knowledge related to brain wounds and the expectation of improving the treatment of brain wounds? If so, how?

[1] Yes, this research has added to knowledge in the area; however, the lack of published data is disquieting. Any comments regarding therapeutic efficacy would be premature!

[2] Not yet—publications have not been forthcoming. They are ready for this, but judgment must be reserved.

[3] Research is adding to the body of knowledge on brain wounds. Expectation questioned. Needs peer review of results.

[4] The work is not completed, but has increased our body of knowledge in the area. Unfortunately there are no publications as yet. Apparently these publications are coming, so it makes things somewhat better.

When these peer-reviewed manuscripts appear, then perhaps their contributions will be more significant.

[5] This work has the prospect of adding to knowledge about brain wounds. The work has so far only been reported in abstract.

Expectations for improving treatment of brain wounds are impossible to evaluate.

[6] Yes, I feel it is adding to [the] information base, especially in area of missile-induced apnea. Due to lack of publications, it is tough to realize expectations. (Perhaps this question could be asked next year.) We were informed during discussions that the PI has three publications about to be published. Time in the open literature will lend itself to knowing if things will be immediately transferred to treatment regimens.

[7] No published data as yet. Expectations [are] difficult to determine.

[8] [No comment]

Third Question: Does the research completed to date attempt to provide information on too many variables?

[1] No!! [Respondent referenced his or her previous answer.] The variables are broad based and may, upon superficial examination, appear diffuse. Yet, again, the nature of the questions asked mandate such an approach. Importantly, the variables assessed show focusing as the application progresses.

[2] [Respondent referenced his or her previous answer.] In view of the paucity of information available, it was appropriate to look at this large group of variables (CBF [Cerebral Blood Flow], CMROs, ICP, etc.). Statistical analysis however, is not discussed to indicate how these variables would be handled. Anything less would probably not have been approved for funding.

[3] No.

[4] [Respondent referenced his or her previous answer.] No. In this type of descriptive proposal, variables must be measured. He has lots of variables, but they are probably all of importance. To correlate the physiological, metabolic, pathologic, and neurological functional variables is important. If he had not said he would make these measurements, we

would criticize him for not doing so. I would like to have seen precisely how all these correlations would be accomplished. This was somewhat deficient in the proposal.

[5] [Respondent referenced his or her previous answer.] The original proposal was exploratory and listed a large number of variables to be evaluated. It certainly outlined an optimistic view of probable accomplishments.

Information has been provided on a number of variables.

[6] No. Sufficient information has been provided on appropriate variables.

[7] Yes.

[8] [No comment]

Fourth Question: Does the research completed to date take an overly simplistic view of a more complex problem?

[1] No!! [Respondent referenced his or her previous answer.] The view is "simplistic"; however, when little is known about the sequelae of the injury, such a simplified approach is valid.

[2] [Respondent referenced his or her previous answer.] Simplified, yes, but <u>not</u> "simplistic."

[3] No—[questions] 3 and 4 are valid only because of distinction between proposed and performed.

[4] [Respondent referenced his or her previous answer.] This issue is a very complex one, which the PI probably understated in the proposals. I think he attempted to come up with the simplest model possible.

[5] Second proposal (1985) better focused than original. More reasonable expectations.

[6] No. It would be helpful to have an overall view of the research plan to support it, however. [Respondent referenced his or her previous answer.] In spite of the many variables (and in hindsight), I feel the PI oversimplified the problem. His approach of going with a sphere rather

than a multishaped fragment—I think it was done appropriately. It at least offered a model of reproducibility and predictability.

[7] [Respondent referenced his or her previous answer to question 3. "Does the research to date attempt to provide information on too many variables?] Yes.

[8] [No comment]

Fifth Question (a): Do the researcher's reports filed to date clearly and specifically indicate, for each type of experiment, the methodology used to interpret/analyze the data?

[1] The yearly reports report, in exhaustive detail, the methods used and the plan of data analysis.

[2] Adequately outlined in progress report.

[3] Much better than proposed.

[4] I still think the data analysis sections, biostatistical analysis, and correlational analysis leave something to be desired.

[5] The progress reports are quite detailed and give considerable detail.

[6] Yes. (If you include recently added information).

[7] [No comment]

[8] [No comment]

Fifth Question (b): Do the researcher's reports filed to date clearly and specifically indicate, for each type of experiment, a step-by-step description of the research methods/procedures used?

[1] Again, the yearly reports provide considerable detail on methods and procedures.

[2] Yes.

[3] Much better than proposed.

[4] Yes. I think the PI has provided very detailed progress reports, describing what was done.

[5] [No comment]

[6] No. The excerpts from notebooks are very good. However, there appears to be a lack of specific protocol sequencing and procedures.

[7] [No comment]

[8] [No comment]

Fifth Question (c): Do the researcher's reports filed to date clearly and specifically indicate, for each type of experiment, the number of animals used?

[1] Adequate detail is provided. Moreover, the trend toward a reduction in animal numbers is viewed as a positive feature.

[2] They have scaled down [the number of] original animals required. This is appropriate.

[3] Much better than proposed. Seems to be generally prudent in the use of animals based on research findings.

[4] Yes. He (PI) has listed all the animals utilized. He is modifying the animal numbers utilized.

[5] A reasonable description of successful (data-generating) experiments are given in progress reports. A more explicit [thought not completed].

[6] Yes. In fact, animal numbers appear to be on the decline.

[7] [No comment]

[8] [No comment]

Sixth Question: Are the treatment drugs the researchers currently plan to test likely to provide information immediately transferable to humans with penetrating brain wounds?

[1] Perhaps! If highly successful, they may form the basis of a new human clinical trial.

[2] Appropriate choice of drugs based on current ideas about head injury. Without a study of dose response it is difficult to immediately extrapolate the results.

[3] Low probability of clinically significant finding.

Opiate antagonist may give results that prompt further studies.

Low probability of success does not negate the research.

[4] Yes. I believe whatever information, either positive or negative, coming from these data would be immediately transferable to humans. The question is whether it is likely that he'll find something. But all you need is one drug that works.

[5] The value [thought not completed].

[6] These are a reasonable group of drugs and include those under current review. Whether or not they would have immediate impact is debatable. The potential for immediate transfer to humans is there, especially if a drug is proven especially good or bad.

[7] At least one of the agents is epileptogenic—"likely" and "immediately"—makes this question difficult to answer.

[8] [No comment]

Seventh Question: Is it appropriate to test non-FDA-approved treatment drugs in this research?

[1] Yes!

[2] Of course. How else can you get FDA approval for human use?

[3] Yes. FDA approval can follow.

[4] Yes. But how silly this question is. One needs to test drugs first on animals to obtain FDA approval. If this is an anti-vivisectionist-motivated question, then it's a ridiculous one. I suppose they want these drugs tested on people first.

[5] Certainly—FDA approval of drugs would require evaluation in an animal model.

Only FDA-approved drugs would eliminate testing of new agents.

[6] Yes.

[7] Yes.

[8] [No comment]

Eighth Question: Is a valid neurological rating scale being used?

[1] As originally proposed, the neurological rating scale was totally inadequate. However, as the research has progressed, clear improvement in the rating scale has been achieved. This revised scale appears more appropriate and may allow for the testing of the chosen therapeutic approaches. Caution should be exercised that the use of cannula (arterial) may complex neurological assessment!

[2] One has been added since the original proposal.

[3] One used in practice is much improved on [that] proposed. Further improvements might improve sensitivity of drug experimental evaluations.

Question confounding factor RE multiple anesthetics. Question effects of drugs on observations.

[4] The original neurological examination was poorly devised. The newer proposed beam examination is a much more sensitive test. Animals with catheters may show impaired response and the PI should consider validating this response. So there are potential problems with this newer neurological examination.

[5] The proposal as originally written did not have an adequate neurological evaluation. The latest progress report does give a much more sophisticated set of neurological observations for evaluation of injury and recovery.

[6] In the original proposal, perhaps too inadequate and simplistic. Animals with multiple catheters in multiple sites may not have the desire for movement. The original rating scale was incomplete and, for me, tough to interpret data.

In the last progress report, a refined, more applicable, rating scale was presented. This is a vast improvement.

[7] Multiple catheters in limbs makes motor skills difficult to evaluate. Post-op[erative] pain is going to contribute to the neurological exam.

[8] [No comment]

Ninth Question (a): Is there evidence in the reports on the research completed to date that the researcher is relating his findings to the existing body of knowledge for each type of experiment?

[1] Adequate.

[2] [No comment]

[3] Generally integrating current methods.

Question other research that is important to relate to since he is exploring new boundaries.

[4] In the progress report write-ups, he has related his work to the literature; however, it is difficult to interpret because he has no publications from which we can determine.

[5] The basic findings on the effects of injury upon physiological and biochemical [sentence unfinished].

[6] Yes, he is continually updating research effort as new information arises, and this is reflected in changing methodologies.

[7] Yes.

[8] [No comment]

Ninth Question (b): Is there evidence in the reports on the research completed to date that the researcher is relating his findings to the existing body of knowledge for work in forensics, military science, law enforcement, and recreational firearms pertaining to the shape, size, weight, and velocity of the missile and characteristics of injuries they cause?

[1] Adequate.

[2] [No comment]

[3] Importance outweighed by need to develop consistent model.

[4] I do not think he has done this very well, particularly for forensics, law enforcement, etc., but this is not within the scope of this grant.

[5] This is not applicable to the aims and purposes of the study.

[6] No, but adequately covered important data relating to research.

[7] No.

[8] [No comment]

Tenth Question: Overall, how would you rate the goals/methods and value of research completed to date?

	5 Very high	4 Somewhat high	3 Neither low nor high	2 Somewhat low	1 Very low
1.		X			
2.			X		
3.		X			
4.			X		
5.			X		
6.		X			
7.				X	
8.	X				

[1] [No comment]

[2] [No comment]

[3] [No comment]

[4] Only because he hasn't published. When the papers appear, I would move him to the "somewhat high."

[5] The primary fault to date is the [sentence unfinished].

[6] [No comment]

[7] [No comment]

[8] [No comment]

II. Experimental Model

A. Research as Proposed

First Question (a): Has the researcher developed a valid model for the investigation of battlefield brain injury in terms of the way the injury is inflicted?

[1] Given the constraints inherent in modeling this issue and the need to produce a reproducible injury amenable to testable therapeutic intervention, the model seems adequate.

[2] Adequate for missile injury—"battlefield" issue cannot be addressed nor should it.

[3] Probably best model available for penetrating brain injury.

[4] This is a valid model of brain (missile) injury which may occur on the battlefield. No injury is perfect. This one isn't either, but I believe it's the best kind of model to simulate battlefield injury available.

[5] The experiments are designed to make a model. It does replicate one kind of injury that may occur on a battlefield.

[6] Yes, this is an approximation of the type of injury that might occur on a battlefield.

[7] [Respondent referenced his or her previous answer.] There is no good "battlefield" injury model, but this is as good an approximation as is available.

[8] [No comment]

First Question (b): Has the researcher developed a valid model for the investigation of battlefield brain injury in terms of planned application of the findings?

[1] The application of the findings appears appropriate.

[2] This is the driving force of the study—to develop application of treatment. It will depend on the results that have yet to be obtained.

[3] Depends on results and extrapolation needed.

[4] [No comment]

[5] The model proposes to test certain variables. Some are valid considerations for battlefield therapies.

[6] Yes, this can be applied to the battlefield scenario.

[7] [No comment]

[8] [No comment]

Second Question: Could the research objectives, if valid, be achieved using alternatives to animals?

[1] No.

[2] No.

[3] No. In the future, modeling techniques and other data can be used in an adjunctive manner. [Refinement, replacement, reduction]

[4] No! This research can be done only in animals. There are no other alternative techniques available which could give the same data. Absolutely not.

[5] In no way—there is not sufficient understanding of brain response to injury to predict the variables involved.

There is a lack of controlled data on vasomotor and hormonal responses to injury of brain tissue.

The basis of factors influencing survival of neuronal tissue under compromised conditions are not adequately understood.

[6] No, [not at this time] (although future studies may lend themselves to applying alternative strategies: [such as] reduction, replacement or refinement.)

[7] Yes, there is a host of human data available—not addressing every aspect of the research, but it is an avenue that should be looked at as a way to reduce the number of animals used. Computer models are becoming more available in missile injuries and should be considered.

[8] [No comment]

Third Question: If only an animal model can be used, is the cat the most appropriate animal model?

[1] The model appears appropriate and well-defended and well-justified by the applicant. Although the cat does have some limitations in terms of neuraxial alignment and its nonpurpose breed nature, no alternative animal models could be identified.

[2] Well justified on p. 85, volume 1 [of binder reviewed by medical panelists; see app. II "Rationale for Using Cats," excerpt from second proposal].

[3] Cat is supported as good experimental model. Issues: Physiology—cerebral blood flow, opiate handling, neurons.

[4] The cat is an appropriate model for this study. There is much work in the literature already on cats and in other head injury models.

[5] Given the various considerations of size, availability, brain configuration, cost, and background information—the cat is a good compromise.

[6] The most appropriate model is probably the primate. The PI went down the other lower species in a discussion section of an annual report and [the] cat is the best, [the] PI argues in final report very adequately.

It should be noted that, in the original proposal, the PI justified the use of cats for the wrong reasons. He should have included the above-mentioned discussion rather than describing cats as having "ample white matter, are small, and will not require large and expensive radioisotope doses. They're relatively inexpensive."

[7] [Respondent referenced his or her previous answer.] Yes, there is a host of human data available—not addressing every aspect of the research, but it is an avenue that should be looked at as a way to reduce the number of animals used. Computer models are becoming more available in missile injuries and should be considered.

[8] [No comment]

Fourth Question: Using the selected model, will the research results be transferable to humans? If not, or only partially so, what will be their limitations?

[1] Perhaps.

[2] Perhaps. This model may suggest appropriate choices for preliminary human studies. This question can be answered only after the studies have been done.

[3] Result-dependent. Cannot answer. Model gives insight into planning human research.

[4] Insofar as any model using animals is transferable to humans, so is this one. It is difficult to transfer information from animals to humans, but this is true of all animal experimentation.

[5] In part—as in all models, the details will fit certain human circumstances and in others may not. Even human models do not [thought not completed].

[6] Yes. As to the degree of how much data is transferable [as] is true with any model. As the PI has not published on the research, it is unclear at this time what will be transferable.

[7] Transferring animal data into the human situation is always a problem. Unfortunately, until it has actually been done, we cannot tell whether or not it will work in the "human model" as it has in the animal model.

[8] [No comment]

Fifth Question: If you were assessing the research at the time it was proposed, overall, how would you rate the suitability of the animal model for the proposed research?

	5 Very high	4 Somewhat high	3 Neither low nor high	2 Somewhat low	1 Very low
1.		X			
2.			X		
3.			X		
4.	X				
5.		X			
6.		X			
7.				X	
8.		X			

[1] [No comment]

[2] [No comment]

[3] [No comment]

[4] [No comment]

[5] [No comment]

[6] [No comment]

[7] [No comment]

[8] [No comment]

B. Research as Performed

First Question: Is there evidence in the reports on the research completed to date that the researcher is relating his findings to the existing body of knowledge for the animal model used and for animal models used in similar research?

[1] Yes!

[2] Yes.

[3] Yes.

[4] I think the PI has done this well in his literature reviews of the areas of the grant. Also, we've answered this before.

[5] The investigator is relating his observations to available information in a sound scholarly way. The results have been reported at medical meetings and detailed reports are in press. His literature reviews are comprehensive.

[6] Yes.

[7] Yes.

[8] [No comment]

Second Question: When compared to the results of other research on this subject, are the reported "new findings," such as reversible apnea, unique to the animal model chosen?

[1] Interesting, but not unique!

[2] Although other models of GSW have not been used, apnea has been long noted in GSW.

[3] Not unique to species.

[4] This "apneic" finding is not unique to cats. It occurs in humans and has been demonstrated in the past. So the finding of apnea is not unique and not new!

[5] Certainly not.

Apnea is reported in human beings after head wounds. Question new findings but certainly emphasized here.

[6] Yes (although during the discussions, it was remarked that this may be a reemphasis.)

[7] Not new, not unique to cat. Reported as early as 1894.

[8] [No comment]

Third Question: How would you rate the overall suitability of the experimental model for the research completed to date?

	5 Very high	4 Somewhat high	3 Neither low nor high	2 Somewhat low	1 Very low
1.		X			
2.		X			
3.		X			
4.	X				
5.		X			
6.	X				
7.				X	
8.	X				

[1] [No comment]

[2] [No comment]

[3] [No comment]

[4] This cat model is a good one!

[5] [No comment]

[6] PI adequately presents the spectrum of animal models available to study (e.g., dogs, primates, cats, and rodents) and cat fits best.

[7] [No comment]

[8] [No comment]

III. Animal Care

A. Research as Proposed

First Question (a): Did the proposals clearly and specifically indicate the procedures for operative/postoperative care, with a complete listing of medications, nutrition, and fluids, where appropriate.

[1] Detail on these issues is quite inadequate. It is obvious that, as the investigation proceeds, more attention to detail in this area has developed. However, in the present format, it is difficult to determine precisely what management strategies will be employed!

[2] Incomplete description of protocol.

[3] Poor detail.

[4] It is unclear about which anesthetic in which dose was used in each animal. The present anesthetic protocol with cats (Torbati) pentobarbital appears OK. However, within these protocols, Brevital, pentobarbital, and isoflurane are discussed as being utilized. One area of importance is why they use three different anesthetics: pentobarbital, Brevital, and isoflurane in all their protocols. Are anesthetics variable?

[5] Listings as given are typical for a proposal. The actual control of details of the care should be in the annual use committee of the local institution. Records do not allow evaluation for early experiments. Present data for pentobarbital examples appear adequate in terms of anesthesia. No details [are] available on nutrition and fluids postoperatively.

[6] Records don't allow for evaluation of early experiments.

Anesthetic protocols were inconsistent and appear to differ. Post-op chronic animal management was questionable due to who monitored and when. Are records available? As LSU is an AAALAC-accredited facility, I feel confident that the animals were cared for very well. Clarification needs to be given about post-op analgesics or justification provided for not giving them.

[7] Deferred to other experts on panel.

[8] "Complete" not possible.

First Question (b): Did the proposals clearly and specifically indicate the procedures for the euthanasia methods that would be used and their compliance with ILAR guidelines?

[1] [No comment]

[2] [No comment]

[3] Unable to judge.

[4] Question about decapitation of cats. This needs to be clearly and soundly justified appropriately. The PI wants to do decapitation presumably because of metabolism measurements, but is this appropriate, to make metabolic measurements? Heads decapitated and plunged into liquid N_2 [nitrogen] may not be fast enough to obtain and interpret metabolic data.

[5] Question decapitation. How were animals pretreated?

[6] Proposal #1: Year (1) Yes—administering barbiturates then exsanguination. Year (3) appears to be yes as cats will be tranquilized (with phencyclidine) prior to decapitation. It was unclear how cats were to be decapitated and with what instrument. Information not available about authorization by ACUC [Animal Care and Use Committee] to decapitate cats. Proposal #2: Yes— IV barbiturates and decapitation or IV KCI.

[7] Deferred to other experts on panel.

[8] Question guillotine.

First Question (c): Did the proposals clearly and specifically indicate the procedures for veterinary support staff and trained ancillary personnel to provide care for the animals?

[1] Yes!

[2] Not evident from protocol.

[3] Appears adequate.

[4] I would like to know more about post-op care. Who checks physiological parameters, i.e. heart rate, blood pressure, pupillary vasodilation. This is critical for pain in these animals. How is "pain" monitored and

whether analgesics are necessary. I'm also concerned about post-op care and evaluations—who observes water and food intake, urine and fecal output? Can animals groom themselves appropriately? Can they exercise properly? etc. It is important to know who does this and whether charts and records are kept on these daily measurements.

[5] AAALAC-approved.

[6] Due to fact that LSU is an AAALAC-accredited facility, I assume the LSU veterinary support staff are adequately trained to provide care. LSU has an Assurance on file with OPRR [Office for the Protection From Research Risks] and is both AAALAC-accredited and in good standing. This Assurance states that the attending veterinarian oversees animal care and use. I must, therefore, assume ancillary personnel are adequately trained. Otherwise something would have been noted.

[7] Deferred to other experts on panel.

[8] [No comment]

Second Question (a): Were ILAR/AAALAC guidelines for the housing of the animals addressed in the proposal, specifically in terms of surgical and postoperative care facilities?

[1] Although AAALAC guidelines have been satisfied, more detail would seem desirable.

[2] Assumed because of AAALAC facility.

[3] [No comment]

[4] [No comment]

[5] [No comment]

[6] Due to the fact that LSU is an AAALAC-accredited facility and is in good standing with the Office for the Protection From Research Risks of the Public Health Service. I must assume all of these (a-d) are in compliance with the Guide for the Care and Use of Laboratory Animals.

[7] Cannot adequately answer these questions with the data given.

[8] Beyond ILAR/AAALAC guidelines, no.

Second Question (b): Were ILAR/AAALAC guidelines for the housing of the animals addressed in the proposal, specifically for separation of noncompatible animals?

[1] [No comment]

[2] Assumed because of AAALAC facility.

[3] [No comment]

[4] [No comment]

[5] [No comment]

[6] Due to the fact that LSU is an AAALAC-accredited facility and is in good standing with the Office for the Protection From Research Risks of the Public Health Service. I must assume all of these (a-d) are in compliance with the Guide for the Care and Use of Laboratory Animals.

[7] Cannot adequately answer these questions with the data given.

[8] [No comment]

Second Question (c): Were ILAR/AAALAC guidelines for the housing of the animals addressed in the proposal, specifically for noise control?

[1] [No comment]

[2] Assumed because of AAALAC facility.

[3] [No comment]

[4] [No comment]

[5] [No comment]

[6] Due to the fact that LSU is an AAALAC-accredited facility and is in good standing with the Office for the Protection From Research Risks of the Public Health Service. I must assume all of these (a-d) are in compliance with the Guide for the Care and Use of Laboratory Animals.

[7] Cannot adequately answer these questions with the data given.

[8] [No comment]

Second Question (d): Were ILAR/AAALAC guidelines for the housing of the animals addressed in the proposal, specifically for exercise facilities?

[1] [No comment]

[2] Assumed because of AAALAC facility.

[3] [No comment]

[4] [No comment]

[5] [No comment]

[6] Due to the fact that LSU is an AAALAC-accredited facility and is in good standing with the Office for the Protection From Research Risks of the Public Health Service. I must assume all of these (a-d) are in compliance with the Guide for the Care and Use of Laboratory Animals.

[7] Cannot adequately answer these questions with the data given.

[8] [No comment]

Third Question: If you were assessing the research at the time it was proposed, overall, how would you rate the quality of the proposed care for animals to be used in the research?

	5 Very high	4 Somewhat high	3 Neither low nor high	2 Somewhat low	1 Very low
1.			X		
2.		X			
3.				X	
4.			X		
5.			X		
6.		X			
7.					
8.		X			

[1] [No comment]

[2] [No comment]

[3] Generally poorly described proposals.

[4] I need to have more information. I think it's OK, but I have questions which must be answered. [Respondent referenced his or her previous answer.] I would like to know more about post-op care. Who checks physiological parameters, i.e. heart rate, blood pressure, pupillary vasodilation This is critical for pain in these animals. How is "pain" monitored and whether analgesics are necessary. I'm also concerned about post-op care and evaluations—who observes water and food intake, urine and fecal output? Can animals groom themselves appropriately? Can they exercise properly? etc. It is important to know who does this and whether charts and records are kept on these daily measurements.

[5] [No comment]

[6] [No comment]

[7] [Did not check a category and had no comment.]

[8] [Respondent referenced his or her previous answer.] If in accordance with ILAR/AAALAC.

B. Research as Performed

First Question: In the research completed to date have appropriate procedures been used for operative/post operative care, including the use of medications, nutrition, and fluids where appropriate?

[1] Detail is again inadequate. One is not confident of precisely what is being done. The experiments performed to date appear to employ appropriate operative and postoperative care; however, more detail is again required!

[2] [No comment]

[3] Poor records.

[4] [No comment]

[5] Information is inadequate to judge.

[6] Anesthesia use was inconsistent as to agents and justification for use or nonuse. Post-op care information not complete.

Although fluid intake was to be monitored and provided for when cats did not drink, there was no mention of how to provide for caloric needs. As most species exhaust glycogen stores within 24 hours when not eating, an animal which did not eat for 2-3 days could very well be ketoacidotic.

Procedures for the management of chronic animals not provided. Animals records (what we reviewed from the notebooks) do not reflect post-op care.

[7] Deferred to other experts on panel.

[8] Pretty good. But the anesthetic should be more clearly defined.

Second Question: Has the research completed to date adequately provided for veterinary support staff and trained ancillary personnel to care for the animal used?

[1] Yes.

[2] AAALAC facility.

[3] Appears so.

[4] [No comment]

[5] Given the ILAR and AAALAC approved facility [status of LSU], one presumes so, but a more direct set of data is needed for a sound judgement.

[6] [Respondent referenced his or her previous answer.] Due to the fact that LSU is an AAALAC-accredited facility, I assume the LSU support staff are adequately trained to provide care. LSU has an Assurance on file with OPRR and is both AAALAC-accredited and in good standing. This Assurance states that the attending veterinarian oversees animal care and use. I must, therefore, assume personnel are adequately trained. Otherwise something would have been noted.

[7] Deferred to other experts on panel.

[8] [Respondent referenced his or her previous answer.] Pretty good. But the anesthetic should be more clearly defined.

Third Question (a): Has the research completed to date adequately provided for the housing of the animals used, specifically in terms of surgical and postoperative care facilities?

[1] [No comment]

[2] [No comment]

[3] [No comment]

[4] [No comment]

[5] Not documented, but approved facility and procedures suggest that they may have been.

[6] Anesthesia variability may interfere with the data. [Respondent referenced his or her previous answer.] Due to fact that LSU is an AAALAC-accredited facility, and in good status with the Office for the Protection From Research Risks of the Public Health Service. I must assume all of these (a-d) are in compliance with the Guide for the Care and Use of Laboratory Animals.

[7] Deferred to other experts on panel.

[8] [No comment]

Third Question (b): Has the research completed to date adequately provided for the housing of the animals used, specifically in terms of separation of noncompatible animals?

[1] [No comment]

[2] [No comment]

[3] [No comment]

[4] [No comment]

[5] Not documented, but approved facility and procedures suggest that they may have been.

[6] [Respondent referenced his or her previous answer.] Due to fact that LSU is an AAALAC-accredited facility and in good status with the Office for the Protection From Research Risks of the Public Health Service. I must assume all of these (a-d) are in compliance with the Guide for the Care and Use of Laboratory Animals.

[7] Deferred to other experts on panel.

[8] [No comment]

Third Question (c): Has the research completed to date adequately provided for the housing of the animals used, specifically in terms of noise control?

[1] [No comment]

[2] [No comment]

[3] [No comment]

[4] [No comment]

[5] Not documented, but approved facility and procedures suggest that they may have been.

[6] [No comment]

[7] Deferred to other experts on panel.

[8] [No comment]

Third Question (d): Has the research completed to date adequately provided for the housing of the animals used, specifically in terms of exercise facilities?

[1] [No comment]

[2] [No comment]

[3] [No comment]

[4] [No comment]

[5] Not documented, but approved facility and procedures suggest that they may have been.

[6] [No comment]

[7] Deferred to other experts on panel.

[8] [No comment]

Fourth Question: Have the euthanasia methods used in research completed to date conformed to ILAR guidelines?

[1] Yes.

[2] [No comment]

[3] Unable to tell.

[4] [No comment]

[5] As per information provided—yes, but details were skimpy.

[6] [Respondent referenced his or her previous answer.] Proposal #1: Year (1) Yes—administering barbiturates then exsanguination. Year (3) appears to be yes as cats will be tranquilized (with phencyclidine) prior to decapitation. It was unclear how cats were to be decapitated and with what instrument. Information not available about authorization by ACUC

[Animal Care and Use Committee] to decapitate cats. Proposal #2: Yes—IV barbiturates and decapitation or IV KCI.

[7] I am concerned about the decapitation model.

[8] [No comment]

Fifth Question: Overall, how would you rate the quality of care given to the animals used in the research completed to date?

	5 Very high	4 Somewhat high	3 Neither low nor high	2 Somewhat low	1 Very low
1.			X		
2.		X			
3.			X		
4.			X		
5.		X			
6.		X			
7.					
8.	X				

[1] [No comment]

[2] As can be determined from material submitted.

[3] Poor post-op records.

[4] But I would like to see more data. I need more information about how this care is given.

[5] Probably, but more details needed—investigator seemed sensitive to animal care issues.

[6] [No comment]

[7] [Did not check a category and had no comment]

[8] If in conformance [with AAALAC].

IV. Anesthetic Controls

A. Research as Proposed

First Question: Were the proposed anesthetic controls adequate to protect the animals from pain during the experimental procedures?

[1] As proposed, detail is limited. In general, the controls seem adequate.

[2] [No comment]

[3] Poor proposals. Not explicit.

[4] The anesthetics utilized are OK. But the use of several anesthetics here is confusing. Why use Brevital, pentobarbital, and isoflurane? How will these anesthetics and/or the combinations of anesthetics affect data interpretation? This point is unclear and should be addressed by the PI.

[5] Questioned [the use of] nitrous oxide [as an anesthetic]. In general, procedures actually used were reasonable, but details to make a sound judgment were not provided for some experiments.

[6] Generally, yes. There was a problem in drawing analogies from humans to cats. Nitrous oxide used alone does not provide adequate analgesia in cats, whereas the human dental experience with the drug seems desirable.

Proposal does not describe those animals which will be chronically maintained. Post-op analgesia was not specified.

Initially, the PI proposed to use phencyclidine to "tranquilize" the animal. This dissociative anesthetic was a poor choice, as it causes hypertension, hypotension, bradycardia, decreased control venous pressure, etc. I was delighted to see he didn't use it.

[7] [No comment]

[8] Real issue as to the variety of anesthetics used.

Second Question: Were the proposed medications adequate to protect the animals from pain during the postoperative recovery period?

[1] [No comment]

[2] [No comment]

[3] Not very powerful. No drugs given.

[4] There is an important general question here of overall post-op care of animals. One really requires an ICU to care for these animals. Precisely how this will be done is unclear. Who cares for these animals. [Respondent referenced his or her previous answer.] I would like to know more about post-op care. Who checks physiological parameters, i.e. heart rate, blood pressure, pupillary vasodilation This is critical for pain in these animals. How is "pain" monitored and whether analgesics are necessary. I'm also concerned about post-op care and evaluations—who observes water and food intake, urine and fecal output? Can animals groom themselves appropriately? Can they exercise properly? etc. It is important to know who does this and whether charts and records are kept on these daily measurements.

[5] Based upon human experiences, postoperative pain should be minimal in the protocol used.

[6] The postoperative management of chronic animals was unclear with respect to provision for animals' pain postoperatively. If analgesic would interfere with science and could not be provided, a justification to that effect would have been helpful. Furthermore, some endpoint should have been provided so as not to withhold analgesics indefinitely if they were indicated.

[7] [No comment]

[8] [No comment]

Third Question: Would the proposed anesthetic controls and other medications have affected the research results?

[1] [No comment]

[1] [No comment]

[2] [No comment]

[3] Vague.

[4] [No comment]

[5] [Did not check a category and had no comment]

[6] [No comment]

[7] [Did not check a category and had no comment]

[8] [No comment]

B. Research as Performed

First Question: In the research completed to date, have adequate anesthetic controls been used to protect the animals from pain during the experimental procedures?

[1] [No Comment]

[2] [No comment]

[3] Apparently so.

[4] [Respondent referenced his or her next answer.] All anesthetics may affect outcome from neural injury. Pentobarbital has been shown to be effective in head injury and in ischemia. Its effectiveness in ischemia, however, has come under question. Other anesthetics, like isoflurane or halothane, have also been implicated in altering neural outcome from injury. This is a difficult question to address and unless specific outcome studies are performed to determine the effects of anesthetics, I believe this question will go unanswered. The PI, however, should have dealt with this potential problem in the text of the proposal.

[5] From the protocols provided, adequate anesthesia appears to have been used.

[6] Pre-op and intra-op: Yes (in spite of anesthetic irregularities). Post-op information not available.

[7] [Respondent referenced his or her previous answer.] While I defer to Panelist # 6, because of his expertise in animal medicine, I have grave concerns as to whether or not the animals, especially in the post-op period, experienced pain to their head wounds, raised ICP (which does cause headache—ask anyone with psuedotumor cerebri), and their wound sites for catheter placement.

[8] [No comment]

Third Question: Did the type of anesthesia and other medication used affect the results of the research completed to date?

[1] The variety of anesthetics and their possible interaction does raise several problems. Specifically, the use of multiple agents could complicate data analysis. This situation should require more consideration for more consistent forms of anesthetic use.

[2] Because of the variation in anesthetics used, some questions must be raised as to how this affects results.

[3] Variability [is] a problem. Barbiturates could confound [the research result].

[4] All anesthetics <u>may</u> affect outcome from neural injury. Pentobarbital has been shown to be effective in head injury and in ischemia. Its effectiveness in ischemia however has come under question. Other anesthetics, like isoflurane or halothane, have also been implicated in altering neural outcome from injury. This is a difficult question to address and unless specific outcome studies are performed to determine the effects of anesthetics, I believe this question will go unanswered. The PI however should have dealt with this potential problem in the text of the proposal.

[5] Probably—unavoidable.

[6] The variety of anesthesia (barbiturates versus inhalation) may have influenced acquired data.

[7] [Respondent referenced his or her previous answer.] Yes, barbiturates have a "protective" effect on the brain and in some institutions and in the clinical setting are used as treatment for head injury (in the control

Fourth Question: Overall, how would you rate the anesthetic controls used in the research completed to date for their capability to protect the animals from pain?

	5 Very high	4 Somewhat high	3 Neither low nor high	2 Somewhat low	1 Very low
1.			X		
2.	X				
3.		X			
4.			X		
5.		X			
6.		X			
7.					
8.	X				

[1] [No comment]

[2] [No comment]

[3] Recent records better than early. [Question] Adequate post-op care? No records.

[4] I believe the anesthetic regimen(s) is unclear, as is the post-op analgesia and post-op care aspects of this proposal. Why use three different anesthetics? How will these alter data interpretation? How can one study using one anesthetic be compared to another using a different anesthetic? Even though this is probably not a painful procedure, it is important for the PI to indicate how he will monitor for pain, and what will he do if pain is apparent. Which analgesia will be utilized? When, and under which conditions, will the animals be euthanized because of pain? These questions need to be dealt with in the body of the text.

[5] [No comment]

[6] Based on the human experience from other panel [members], it appears that animals should not experience pain. [Whether] postoperative analgesics to be provided or not provided was not adequately addressed.

[7] [Did not check a category and had no comment]

[8] [No comment]

Second Question: Based on the equipment listings and information contained in the proposals, did the research team have the facilities and equipment needed to do the research as proposed?

[1] Adequate.

[2] From limited impression gained from meeting here—photographs—facilities seem adequate.

[3] Yes.

[4] Yes. Very impressive facilities. He can do the experiments as outlined. I have visited the laboratories, and I must say that space and equipment resources are adequate to perform these studies. Essentially all the equipment necessary to complete these studies is available on site.

[5] Yes.

[6] Based on pictures and testimony of one of panel members, it appears the research team had all they needed to do the research effectively.

[7] From the pictures and lists available, it appears that the facilities and equipment are adequate.

[8] [No comment]

Fourth Question: If you were assessing the research at the time it was proposed, overall how would you rate the adequacy of the research team's facilities and equipment to do the proposed research?

	5 Very high	4 Somewhat high	3 Neither low nor high	2 Somewhat low	1 Very low
1.			X		
2.	X				
3.		X			
4.	X				
5.		X			
6.		X			
7.		X			
8.	X				

[1] [No comment]

[2] [No comment]

[3] [No comment]

[4] [No comment]

[5] [No comment]

[6] [No comment]

[7] [No comment]

[8] [No comment]

B. Research as Performed

First Question: Based on the curricula vitae provided, does the research team have the qualifications needed to do the research completed to date?

[1] [Respondent referenced his or her previous answer.] Although the qualifications of all involved appear adequate to conduct the proposed research, there is one concern regarding the overall low productivity of

[8] [No comment]

Third Question: Based on information provided (i.e., the researcher's diagram of the project as currently planned), does the research team have the qualifications needed to do all of the research planned and add to the current body of knowledge in these areas?

[1] Yes.

[2] [No comment]

[3] [No comment]

[4] I have visited Dr. Carey's laboratory about 4-5 years ago. His facilities are excellent. He has all the equipment required to perform the proposed experiments.

[5] Reasonable competence in most areas.

[6] Yes.

[7] [Respondent referenced his or her previous answer.] From the pictures and lists available, it appears that the facilities and equipment are adequate.

[8] [No comment]

Fourth Question: Based on information provided and the facilities and equipment display boards, does the research team have the facilities and equipment needed to do all of the research planned and to add to the current body of knowledge in these areas?

[1] Yes.

[2] [No comment]

[3] [No comment]

[4] [Respondent referenced his or her previous answer.] I have visited Dr. Carey's laboratory about 4-5 years ago. His facilities are excellent. He has all the equipment required to perform the proposed experiments.

[8] [No comment]

Sixth Question: Overall, how would you rate the research team's qualifications to do all of the research currently planned?

	5 Very high	4 Somewhat high	3 Neither low nor high	2 Somewhat low	1 Very low
1.			X		
2.		X			
3.	X				
4.			X		
5.			X		
6.		X			
7.			X		
8.		X			

[1] [No comment]

[2] [No comment]

[3] [No comment]

[4] [Respondent referenced his or her previous answer.] Here, I am concerned with the lack of productivity of these investigators. However, my fears are somewhat alleviated by the fact that there are several, (3) manuscripts in press in Journal of Neurosurgery.

[5] [No comment]

[6] [No comment]

[7] [No comment]

[8] [No comment]

Eighth Question: Overall, how would you rate the adequacy of the research team's facilities and equipment to do all of the research currently planned?

	5 Very high	4 Somewhat high	3 Neither low nor high	2 Somewhat low	1 Very low
1.		X			
2.		X			
3.	X				
4.	X				
5.		X			
6.		X			
7.		X			
8.	X				

[1] [No comment]

[2] [No comment]

[3] [No comment]

[4] [No comment]

[5] [No comment]

[6] [No comment]

[7] [No comment]

[8] [No comment]

VI. Comments on Other Aspects of the Research

[1] In general, the detail provided in the research section of this application is rather limited! This is particularly so in regards to animal management and anesthetic. Clearly written and developed research plans, complemented by considerations of any pitfalls as well as a clear plan of data analysis, would have been helpful. Similarly, details on the precise fashion of anesthesia and management in each experimental paradigm would have helped place the application in a more scientifically rigorous framework.

abstracts of such presentations. Until full review by the scientific/medical community can be made from published material, the results will be of no value and the validity in question.

[6] Productivity: It appears that the PI was a bit overzealous in anticipated accomplishments initially. It does appear that the investigator did a lot of important work. It's unfortunate that publications during this period were not produced. From information available clarification should be given about whether "contracts" are "contracts" or "grants."

As a result of this review panel's efforts, I conclude that the requirements for accountability and methodologies in biochemical research have changed (in just the past 5 years)—per telephone conversation with the LSU chairman of the ACUC (with permission from panel's chair) I was assured that Dr. Carey made every attempt to do what's right. Even before submitting his proposal to the Animal Care Committee, he invited all committee members to review the entire project. The ACUC was comfortable with what is going on and, through their Assurance to NIH, OPRR guarantees to oversee this and all other research efforts at their institutions.

[7] It should be kept in mind that this is a contract and not a grant. The time table set forth in the original proposal was not kept and, apparently, no communication or clarification was offered to the Army in this regard. The protocol itself was changed frequently, again with no apparent justification to the Department of Defense.

Drug testing, while discussed in both protocols, to date (as seen in the information available for review) has not been done.

No publication of the results in a peer-reviewed journal has occurred to date, although we are told some data is soon to be published. It should be kept in mind that the project has been ongoing for 6 years, and one would think that some work would have already been published.

[8] [No comment]

The research is directed at a very important topic that is not being researched elsewhere. It has obvious major importance regarding developing both research questions and testing interventions for use in combat casualty care and for civilian GSW injuries. The relationship between the model and these types of injuries needs to be defined. The availability of a testing model may be very important for testing future therapies. Currently, this is not possible since we cannot describe, let alone control for, case-mix difference (severity) in clinical GSW responses. We await the results of the drug therapies tests.

[4] Yes. I believe this proposal does provide new and important information about the treatment of penetrating brain wounds. First, it has provided a model for future use that is a consistent, graded model of missile injury. The earlier work done in this protocol does add to the body of literature in this area; i.e., edema is not an early problem, lack of autoregulatory CO_2 [carbon dioxide] and O_2 [oxygen] responses of the cerebral circulation. The protocol still represents a promissory note-type study since much (all) of the work regarding use of pharmacological agents remains still to be done. It is likely that new information, positive and/or negative, regarding these agents' usefulness will come from these experiments. I suppose that the major accomplishment to date is that the PI has developed a model of missile injury and now is ready to use this model for a variety of treatment modalities.

[5] The essential point is that this research has developed a model for penetrating missile wounds of the brain. The model has been used to examine the influence of a certain kind of wound upon hemodynamic, hydrostatic, and biochemical changes and to relate these to morbidity and mortality. Some of the observations on the model have provided new emphasis upon the importance of changes previously noted (apnea), while others have shown that other alterations hypothesized to be important appear to be of minor significance (edema). In the sense the work has led to better understanding of the dynamics of a penetrating brain wound and possible ways to enhance therapy, its greatest potential for treatment improvement is the means it gives for systematically evaluating any existing or proposed therapeutic action or agent.

[6] I feel that based on the data presented, I have a better appreciation for the complexity of fragment injury and how it can be distinguished from blunt trauma. The researcher now has a working model ready for drug testing. This panel has found fault with various areas, including lack of statistical plan and anesthetic changes. Overall, however, I feel it was a very worthwhile research effort.

[2] The work is not repetitive. This model has not been used before and treatment paradigms are original with regard to GSWs. The model has established a dose response, i.e., LD_{50} of 1.35 joules, which should prove useful in evaluating treatment.

Since this is the only lab in the country studying GSW (missile injury), it is not duplicative and is an important approach to the problem.

[3] The project does not duplicate existing research. Many of the conclusions to date support or provide experimental detail to improve the understanding of commonly held doctrine and clinical opinion. The research complements previous findings and makes a significant specific contribution in providing a model that others can also use. Future testing of drugs on a standard model could be a significant contribution, even if limited applicability or effect is found.

[4] No. This is essentially the only laboratory in the world working in this area. While there has been some previous work in this area over the years, no other laboratory is active at this time. Thus, the work does not represent duplication of previous work in the area and basically there is no competition with other laboratories at this time.

[5] Some of the research proposed and completed does confirm conclusions reached in uncontrolled, anecdotal reports and in studies on a limited number of observations on monkeys. In part, these represent essential confirmation. Other observations on the model are novel and provide new insights. Most importantly, a number of quantitative measures have given a foundation for evaluating the influence of therapeutic manipulations.

[6] It appears that Dr. Carey has not duplicated existing research. He did validate previous work by Crockard and others en route to his completed research. The panel was provided information as to three publications currently scheduled for near-term release on this research. The published arena will render final decisions about originality.

[7] [Respondent referenced his or her previous answer.] I do not feel that new information has come to light to date. The Crockard data has addressed many of these issues. Clinical papers by Becker, et al. have addressed other of the issues.

The bottom line is, after reading the results of the experiments, has it changed my practice of neurosurgery in the case of gunshot wounds to proposal. Changes with regard to methods, techniques, and ideas are always changing and are based in large part on previous findings. This research still represents useful, important information.

[5] In part, the research does reflect the research proposal—specifically, the development of a model system and some sets of measurements on the model. Many of the experiments proposed, even in the 1983 application, have not yet been reported, e.g., studies using drugs. The projects took new directions in the 1985 application, emphasizing biochemical measures and a new list of therapeutic agents to be tested. Some of the change in direction follow evolution of ideas and approaches in neurosurgical thinking about the possible biochemical bases of brain injury and neuronal death.

The approach to neurological evaluation of the animals has dramatically altered during the second project. This move to a more sophisticated neurological evaluation certainly enhances the value of observation on the effects of agents and pervasive manipulative procedures.

In general, the changes in approach have had salutary effects on the value of the work. They seem to have followed identification of problems or limitations in original approaches.

[6] The research completed to date is somewhat delayed as to what was proposed. In year 3 of the original proposal, the PI was anticipating progressing to drug testing. To date, this has not occurred (to my knowledge).

As the data began to accumulate, the complexity of the problem became more evident. I feel the changes made were acceptable.

The significant changes appear to help establish more information about the model's usefulness prior to drug usage.

[7] Not entirely. Again, this is a contract and not a grant. I personally think that the changes toward a more biochemical approach are more meaningful. [Respondent referenced his or her previous answer.] It should be kept in mind that this is a contract and not a grant. The time table set forth in the original proposal was not kept, and apparently no communication or clarification was offered to the Army in this regard. The protocol itself was changed frequently, again with no apparent justification to the Department of Defense.

not a new finding, reaffirms the idea of airway support immediately following the injury.

[5] The confirmation of a limited role for brain edema and ischemia on mortality from penetrating wounds certainly is useful evidence for treatment. Emphasis on the importance of early apnea as a possible cause of death, prior to triage, is important for emergency care at time of injury, particularly in battlefield conditions. Possible harmful effects of O_2 therapy at time of brain injury is of potential value.

[6] It appears the results based on apnea and support for that may have a renewed emphasis. They may have a direct applicability to humans with penetrating brain wounds.

With respect to changes in therapy regimens for fragment wounds to head, time in the literature (when publications are realized) will tell. It would be useful to verify the apnea occurrence in a higher species (e.g., primates).

[7] No.

[8] The apnea response may well have clinical applicability.

Fifth Question: Which, if any, of the results reported to date are new findings?

[1] Perhaps the most unique and technically new findings center on the course of ICP, CBF [cerebral blood flow], and vaso-reactivity change subsequent to brain wounding. The fact that elevated ICP correlates with intraparenchymal hemorrhage is interesting. Similarly, the fact that these injuries do not result in CBF reductions reaching ischemic levels is of import. Lastly, the fact that the injured brain does not autoregulate or respond to physiological challenge in a normal fashion does provide some useful information which explains the injured brain's increased risk to secondary insult.

[2] Observations on apnea and ICP are not "new," but certainly deserved re-emphasis since they are not widely appreciated.

[3] The "new" findings are more in the level of detail than in a single major revealing finding. To expect the latter would not be reasonable. The data seem to refocus immediate care on airway management of even seemingly lethal head injury. The data provide valuable new detail in

[8] (1) Apnea responses (in spite of Horsley's description), (2) no edema lag response, and (3) no increase in ICP unless hemorrhaging.

Sixth Question (a): At the time the research was initially proposed, if you were on a peer review panel considering whether to fund the research, considering only the information in the researcher's first proposal, how likely would you have been to recommend that the first research proposal be funded?

	5 Very likely	4 Somewhat likely	3 Undecided	2 Somewhat unlikely	1 Highly unlikely
1.				X	
2.	X				
3.				X	
4.		X			
5.		X			
6.		X			
7.		X			
8.	X				

[1] [No comment]

[2] [No comment]

[3] [No comment]

[4] [No comment]

[5] [No comment]

[6] [No comment]

[7] [No comment]

[8] [No comment]

Report From GAO's Medical Panel on Brain-Wound Research Project

This appendix contains the report of GAO's medical panel that met on June 19, 1989. A copy of the panel's report, as it was faxed to GAO by Dr. John A. Jane on October 23, 1989, is provided below.

GAO's Medical Review Panel

In order to review the scientific value of the Army contracts, and to determine if other research of this nature has been done, a medical review panel was convened to review the first and second contracts with Louisiana State University, the quarterly, annual, and final reports to date for each contract, and information on the facilities and equipment used during the research. See appendix for a discussion on how the panel was selected and the individual panel members).

Introduction

Dr. John A. Jane was contacted by Dr. Murray Grant, the Chief Medical Advisor of the General Accounting Office, to participate as Chairman of the expert panel reviewing a Department of Army research project on penetrating head wounds.

The panel consisted of the following members:

John A. Jane, MD. PhD
Professor and Chairman
Department of Neurosurgery
University of Virginia

Howard R. Champion, MD
Chief, Trauma Services
Director, Surgical Intensive Care
Washington Hospital Center

Eugene S. Flamm, MD
Professor and Chairman
Division of Neurosurgery
University of Pennsylvania

Robert F. Hoyt, DVM. MS
Chief, Laboratory Animal Medicine
and Surgery Section
National Heart, Lung, and Blood Institute
National Institutes of Health

Edward R. Perl, MD
Professor and Chairman
Department of Physiology
University of North Carolina

John T. Povlishock, PhD
Professor of Anatomy
Medical College of Virginia

Richard R. Traystman, PhD
Director, Anesthesiology and
Critical Care Medicine
Johns Hopkins School of Medicine

Carrie L. Walters, MD
Neurosurgeon
Phoenix, AZ

In early June, the Chairman and the members of the panel received the research proposals for a contract that ran from 1983 to 1985, as well as a proposal

The panel members thought that the review process was unusual, but valid. Most of the members on the panel were quite familiar with the distinction between grants and contracts, but were more accustomed to either the Study Section-type review of grants where, for example, over a three day period, over 100 grants are reviewed; or, on the other hand, a more traditional site visit in which the site visitors discuss the project with the Principal Investigator. In general, the panel felt that the method used was a valid technique for the review.

Each of the questions included in the booklet entitled "Expert Medical Panel on Brain Injury Research Project" was discussed and a copy of this booklet is appended.

At the end, each panel member recorded his/her impressions of the research effot. The related discussion was open and frank. The Chairman prepared the initial report and circulated it among the panel members who made comments, suggestions, and changes which were then returned to the Chairman. The enclosed document is a summary of this process. At the end of each of the major sections, each panelist confidentially rated the research. The choices and values were: very high = 5, somewhat high = 4, neither low nor high = 3, somewhat low = 2, and very low = 1. The following is a summary of the voting:

Summary of Voting

The assessment of the panel is as follows:

Research at the time it was proposed:

Rated 3.6.

Goals/methods and value of research completed to date:

Rated 3.5.

Suitability of the animal model for the proposed research:

Rated 3.6.

Considering the information in the (1) researcher's final report on work under the first proposal and (2) second proposal, how likely would you have been to recommend that the second research proposal be funded:

Rated 3.4.

The panel members rotated in order to initiate the discussion on each point while the Chairman attempted to focus the discussion on each topic. During and after the completion of the discussion each member prepared written comments. In preparing this report, Dr. Jane attempted to summarize the overall feelings of the group, while also recording individual comments, both positive and negative. What follows then is, first, a section summarizing the five main areas of the evaluation, namely:

1. Research goals, methods, value.
2. Experimental model.
3. Animal care.
4. Anesthetic controls.
5. Investigator's equipment.

Second, concerns taken verbatim from the written comments.

Third, the strengths of the proposal as identified in the write-ups.

1. Research Goals, Methods, Value

The panel concluded that the goals of the research were valid. The problem of missile injury both on the battlefield and in civilian circumstances is important. Understanding the pathophysiology of missile injury is the only way that progress can be achieved in treatment.

A general concern centered on the experimental methods because they were not described with the precision that is usually seen in a research proposal. Most considered this lack of experimental detail as a flaw in the proposal. However, this flaw was not considered to compromise the basic merits of the proposal.

It was strongly felt that progress in improving outcome of brain injury can only be made via studies such as the one proposed.

and, in the reviewer's opinion, a local anesthetic was not enough. The other clinicians on the panel, however, felt strongly that in patients who are shot there is virtually never any report of pain from the bullet wound itself, even with major disruptions of skin, periosteum, and bone.

There was some concern expressed that the anesthetics used (for example barbiturate) might interfere with the study results since they might afford cerebral protection from the wounding. In addition, the panel was concerned with the number of different anesthetics used during the research and the panel was unable to tell how the principal investigator adjusted for these differences. Specifically, it unclear about which anesthetic in which doses was used in each animal.

Each anesthetic creates its own problems in interpretation and the use of a particular anesthetic does not in itself obviate the value of an experiment.

5. Investigator's Equipment

From the information given to us by the GAO, this appears to be excellent.

Dr. Michael Carey is a highly respected member of the neurosurgical community with a long-standing interest in missile injury and a unique clinical experience in the battlefield.

Concerns noted by the panel members:

The Chairman of the panel felt that, in the interests of being totally objective in reporting the findings of everyone, that virtually every negative comment should be included. Eight panel members, with one exception, all felt that this method had the effect of creating a negative impression. Since all, with one exception, felt quite positive about the proposal, there was some question whether or not the comments should be included, but coalesced. The Chairman felt in the interest of objectively reporting the deliberations of the panel that they should be included, but that it should be emphasized that the panel members were objective, demanding, and

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Methodology/Design:

1. Methodological design is weak, information is needed on statistical analysis in working with the many variables, not known how the number of animals is determined for each experiment.

2. Incomplete description of protocol.

3. The number of animals requested is not adequately justified. They strike this reviewer as too high a number for the experimental design.

4. Trials of drugs should include some dose response in order to establish specifications.

5. The hypotheses do not define the goals for the specific treatment paradigms.

6. Methodological design -- overall detail is lacking, problems and pitfalls are not addressed, and a precise plan of data analysis is not given. A plan of statistical analysis is not clearly presented.

7. Original neurological totally inadequate; however, current scale used is more appropriate.

8. Methodological design -- this is one area which was deficient. How the correlation analyses were to be done is unclear. Which statistical analyses are done is not clear. Incomplete statistical analysis. Some of the procedures and methodologies are not well described.

9. Methodological design and step-by-step description of the research is poor. Needs an analysis of the number of animals to be used.

10. Methodological design and step-by-step description of the research is not clear. Statistical significant results -- it is difficult to determine based on information provided.

11. Hypotheses were very broad, and they had been previously looked at in a primate model.

12. Study looked at too many variables.

13. Statistically significant results -- not stat. analysis provided in proposal.

14. Methodological design -- proposal is not clear.

Operative/Postoperative Care:

1. Procedures for operative/postoperative care -- detail on these issues is quite inadequate.

2. More information is needed about postop care in the proposal.

3. Research as performed -- poor records, poor postop records.

4. Postop care information inadequate to judge.

8. The widely noted finding of trauma induced apnea does not appear particularly novel and, indeed, some caution should be exercised when translating these findings in cat to humans sustaining brain wounds. The proposed drug treatment strategies offer the most promise for brain-wounded humans. However, until these drug studies are brought to closure, no comment can be made regarding their applicability to humans.

9. The finding of apnea is not unique and not new.

10. Low probability of clinically significant finding.

11. Does not feel the information gained to date will result in anything of value or that is different than what is already known about in the field of medicine.

Animal Care / Model:

1. Animal care -- poor detail, unable to judge.

2. Believes that research objectives could be obtained without using the "cat model." Believes that computer models could be used. (One reviewer strongly disagreed with this point of view since he felt, as the Chairman did also, that there was a consensus that an animal model had to be used for this type of injury. This point is of course of critical importance and, once again, 7 out of 8 panel members agreed that an animal model was necessary.)

3. Concern about the decapitation protocol.

Other:

1. What were the expectations of the Army? Was it open ended or were there certain performance benchmarks? How was the contract monitored?

While these were the concerns of the panel, the overall feeling was positive. For example. the following were some of the comments made by the panelists:

1. This is an interesting proposal dealing with the an important area of head injury, namely, missile head wounds. While the hypotheses are broadly stated, they are important and clear:..to develop a head injury model which is systematic and reproducible, and in which a variety of therapeutic and pharmacological interventions can be tested. This is of importance and the Principal Investigator has done this well, i.e., his approach is valid.

2. We know little concerning the treatment of gunshot wounds both in military situations and in daily life in our cities; this study can help in this regard.

3. I believe whatever information either positive or negative, coming from these data would be immediately transferable to humans.

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9. I believe that much of the work that is to be done in the future has more applicability than that which has been done already.
10. Important topic with major limitations in our knowledge.
11. Worthwhile, a valid approach.
12. Research follows a systematic plan and valid approach.
13. Research is on track.
14. Research is adding to the body of knowledge on brain wounds.
15. Probably the best model available to penetrating head injury.
16. This is a very important project, funding should continue.
17. The lab's unique research completed to date has made two valuable contributions: 1) developed and characterized a model for ballistic penetrating injury; 2) provided additional detail on the acute changes following injury.
18. The research is a very important topic that has not been researched elsewhere. It has obvious major importance regarding developing both research questions and testing interventions for use in combat casualty care and for civilian gunshot wounds.
19. The project does not duplicate existing research. Many of the conclusions to date support or provide experimental detail to improve the understanding of commonly held doctrine and clinical opinion. The research complements previous findings and makes a significant specific contribution in providing a model that others can also use. Future testing of drugs on a standard model could be a significant contribution, even if limited applicability or effect is found.
20. The current doctrine of combat casualty care could be changed to further emphasize the immediate airway management and head injured battle casualties.
21. Very important to continue funding.
22. I feel.that the hypothesis is both medically valid and important.
23. The PI has developed an important animal model for application for missile injury for both the DOD and the civilian community.

35. In the field of penetrating brain wounds, this investigation must be considered unique. At present, no other group in the nation is conducting such studies. As we are becoming an increasingly violent society with an increased incidence of trauma to the brain, the need for such studies becomes all the more apparent.

Over approximately a six-hour period, a vigorous discussion and analysis of the research project was carried out. The general conclusion was that this is a unique model, that no one else is working in this particular area, and that funding should be continued. A major criticism of each of the reviewers was the lack of productivity. However, abstracts and a Journal of Neurosurgery article may represent the beginning of their productivity. In addition, there was also some question about the exact methodology that was being used. The issue of animal care was addressed. There was general agreement that since this was an accredited AAALAC facility that proper care was being taken. The anesthetic controls were somewhat variable and the criticism that was made focused on validity of results, but not upon whether the animals were protected from pain. It was pointed out that gunshot wounds to the head are not reported by humans who survive to be painful and that however distasteful the appearance of the event may be, conscious appreciation of pain is lost immediately. Moreover, in those humans that survive, pain is not commonly reported when consciousness returns. Everyone agreed that, in the usual site visit situation, the Principal Investigator would have been able to respond to these questions, and that with the technique used for this particular report, this was not possible. One panelist was clearly less enthusiastic on all of these points than the others and these comments have been included in the concerns section. This report has been circulated to all of the panel members and meets with their approval.

Bibliography of Anesthesia Articles Provided by LSU

On November 9, 1989, the principal investigator and others from the LSU Medical School provided us with 41 articles about research using cats and pentobarbital as the anesthesia; the articles are to support the position that pentobarbital is an acceptable anesthetic for use in the LSU project. In the articles that are cited, researchers initiate anesthesia with pentobarbital, but in about one-third (12) of the articles, the anesthesia is maintained over the operative period with nitrous oxide and oxygen. One article discusses the use of pentobarbital in humans; and another discusses the effects of barbiturates. These articles are listed below. In some cases, the text of the articles did not indicate a source, date of publication, or page numbers.

These articles were also reviewed by Dr. Lawrence R. Soma, who emphasized that rigorous control of pentobarbital is essential in a brain injury project such as this one.

Edema and Pentobarbital

Bartko, D., and others. "Effect of Dexamethasone on the Early Edema Following Occlusion of the Middle Cerebral Artery in Cats," pp. 127-37.

Beks and others. "Increase in Intraventricular Pressure in Cold Induced Cerebral Oedema." Acta Physiologica, Pharmacologica, Neerl, Vol. 13 (1965), pp. 317-29. [Anesthesia maintained with nitrous oxide.]

Hatashita, J.C.S., and J.T. Hoff. "Cortical Tissue Pressure in Injured Brain After Subarachnoid Hemorrhages." In Intracranial Pressure VII, J.T. Hoff and A.L. Betz, eds. Berlin, Heidelberg: Springer-Verlag, 1989, pp. 719-21.

Shalit, M.N., and S. Cotey. "Interrelationship Between Blood Pressure and Regional Cerebral Blood Flow in Experimental Intracranial Hypertension." Journal of Neurosurgery, Vol. 40 (May 1974), pp. 594-602.

Go, K.G., J. Gazendam, and A.K. van Zanten. "Influence of Hypoxia on the Composition of Isolated Edema Fluid in Cold-Induced Brain Edema." Journal of Neurosurgery, Vol. 51 (July 1979), pp. 78-84. [Anesthesia maintained with nitrous oxide.]

Long, D.M., and others. "Multiple Therapeutic Approaches in the Treatment of Brain Edema Induced by a Standard Cold Lesion." In Steroids and Brain Edema, H.J. Reulen and K. Schuermann, eds., pp. 87-94.

Flow and Metabolism, Vol. 5 (1985), pp. 241-52. [Anesthesia maintained with nitrous oxide.]

Davis, D.H., and T.M. Sundt. "Relationship of Cerebral Blood Flow to Cardiac Output, Mean Arterial Pressure, Blood Volume, and Alpha and Beta Blockage in Cats." Journal of Neurosurgery, Vol. 52 (June 1980), pp. 745-54.

Stromberg, D.D., and J.R. Fox. "Pressures in the Pial Arterial Microcirculation of the Cat During Changes in Systemic Arterial Blood Pressure." Circulation Research, Vol. 31 (Aug. 1972), pp. 229-39.

Yokoyama, R., and others. "Experimental Study of the Correlation Between Evoked Potentials (SEP and AEP) and the Perfusion Pressure." In Intracranial Pressure VII, J.T. Hoff and A.L. Betz, eds. Berlin, Heidelberg: Springer-Verlag, 1989, pp. 845-49.

Heiss, W., and H. Traupe. "Comparison Between Hydrogen Clearance and Microsphere Technique for rCBF Measurement." Stroke, Vol. 12 (1981), pp. 161-67. [Anesthesia maintained with nitrous oxide.]

Rosner, M.J., and D.P. Becker. "Origin and Evolution of Plateau Waves." Journal of Neurosurgery, Vol. 60 (Feb. 1984), pp. 312-24. [Anesthesia maintained with nitrous oxide.]

Little, J.R. "Modification of Acute Focal Ischemia by Treatment With Mannitol." Stroke, Vol. 9 (Jan.-Feb. 1978), pp. 4-9.

Tanaka, K., and others. "Regional Alterations in Glucose Consumption and Metabolite Levels During Postischemic Recovery in Cat Brain." Journal of Cerebral Blood Flow and Metabolism, Vol. 5, (1985), pp. 502-11. [Anesthesia maintained with nitrous oxide.]

Ginsberg, M.D., W.W. Budd, and F.A. Welsh. "Diffuse Cerebral Ischemia in the Cat: 1. Local Blood Flow During Severe Ischemia and Recirculation." Annals of Neurology, Vol. 3 (June 1978), pp. 482-92. [Anesthesia maintained with nitrous oxide.]

Silver, I.A. "Changes in PO_2 and Ion Fluxes in Cerebral Hypoxia-Ischemia." Advances in Experimental Medical Biology (1977), pp. 299-312.

DeWitt, D.S., and others. "Effects of Fluid-Percussion Brain Injury on Regional Cerebral Blood Flow and Pial Arteriolar Diameter." Journal of Neurosurgery, Vol. 64 (May 1986), pp. 787-94.

Duckrow, R.B., and others. "Oxidative Metabolic Activity of Cerebral Cortex After Fluid-Percussion Head Injury in the Cat." Journal of Neurosurgery, Vol. 54 (May 1981), pp. 607-14.

Zierski, J. "Blood Flow in Brain Structures During Increased ICP." Acta Neurochirurgica, Suppl. 40 (1987), pp. 95-116.

Other Articles

DeSalles, A.A.F., and others. "Transient Suppression of Event-Related Evoked Potentials Produced by Mild Head Injury in the Cat." Journal of Neurosurgery, Vol. 66 (Jan. 1987), pp. 102-08.

Auer, L.M., and others. "Sympatho-Adrenergic Influence on Pial Veins and Arteries in the Cat." In Cerebral Blood Flow: Effects of Nerves and Neurotransmitters, D.D. Heistad and M.L. Marcus, eds. Elsevier North Holland, Inc., 1982, pp. 291-300. [Anesthesia maintained with nitrous oxide.]

Langfitt, T.W., and others. "Contribution of Intracranial Blood Volume to Three Forms of Experimental Brain Swelling." pp. 261-70.

Siejoe, B.K. Brain Energy Metabolism. Chichester: John Wiley and Sons, pp. 237-38.

Table IX.3: Research Data on Intracranial Pressure—Cats Wounded at 0.9 Joules

Time (mins.)	M219	M227	M231	M233	M239	Means	Standard deviation
0	7.0	16	8.0	10	3	8.8	4.764452
1	28.0	46	13.0	19	17	24.6	13.164346
3	34.0	30	14.0	14	18	22.0	9.380832
5	26.0	26	17.0	10	17	19.2	6.833740
10	13.0	24	25.0	8	18	17.6	7.231874
20	9.0	22	29.0	9	32	20.2	10.848963
30	8.0	22	38.5	10	26	20.9	12.471969
60	8.0	20	31.0	11	18	17.6	8.961027
120	7.0	29	29.0	15	23	20.6	9.528903
180	9.0	30	27.5	22	20	21.7	8.167007
240	8.0	30	25.0	22	21	21.2	8.167007
300	10.5	26	21.0	17	20	18.9	5.705261
360	10.5	26	25.0	19	20	20.1	6.168468

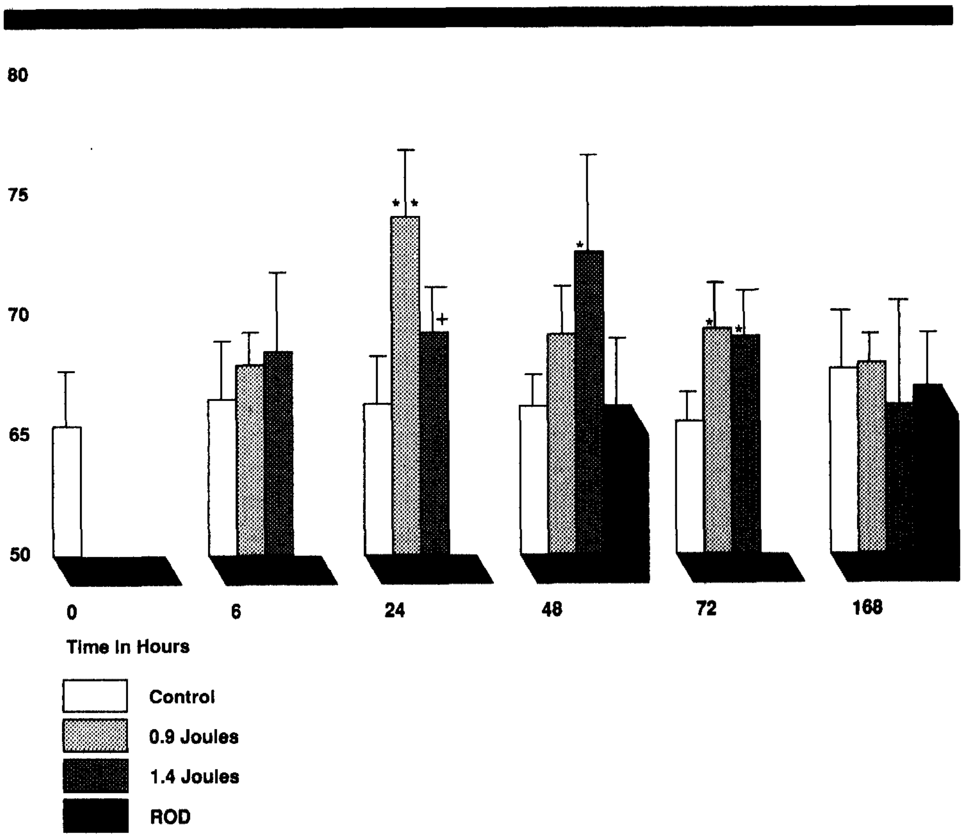
Source: LSU's Final report on the first contract, p. 102.

Table IX.4: Research Data on Intracranial Pressure—Cats Wounded at 1.4 Joules

Time (mins.)	M22	M328	M234	M237	M243	Means	Standard deviation
0	4	11	5	5	8	6.6	2.880972
1	20	20	36	30	78	36.8	24.024987
3	27	19	30	43	106	45.0	35.178118
5	23	17	29	38	72	35.8	21.672563
10	14	18	31	32	56	30.2	16.437761
20	18	21	33	37	36	29.0	8.860023
30	19	22	33	35	35	28.8	7.694154
60	20	20	40	23	66	33.8	19.829271
120	20	32	35	15	51	30.6	14.081903
180	17	34	27	16	51	29.0	14.370108
240	22	36	26	30	55	33.8	12.930584
300	24	40	28	36	52	36.0	10.954451
360	28	35	31	36	47	35.4	7.231874

Source: LSU's final report on the first contract, p. 103.

Figure IX.1: Brain Water in the White Matter of the Cerebral Hemisphere for Control Cats and Cats Injured at Different Energy Levels

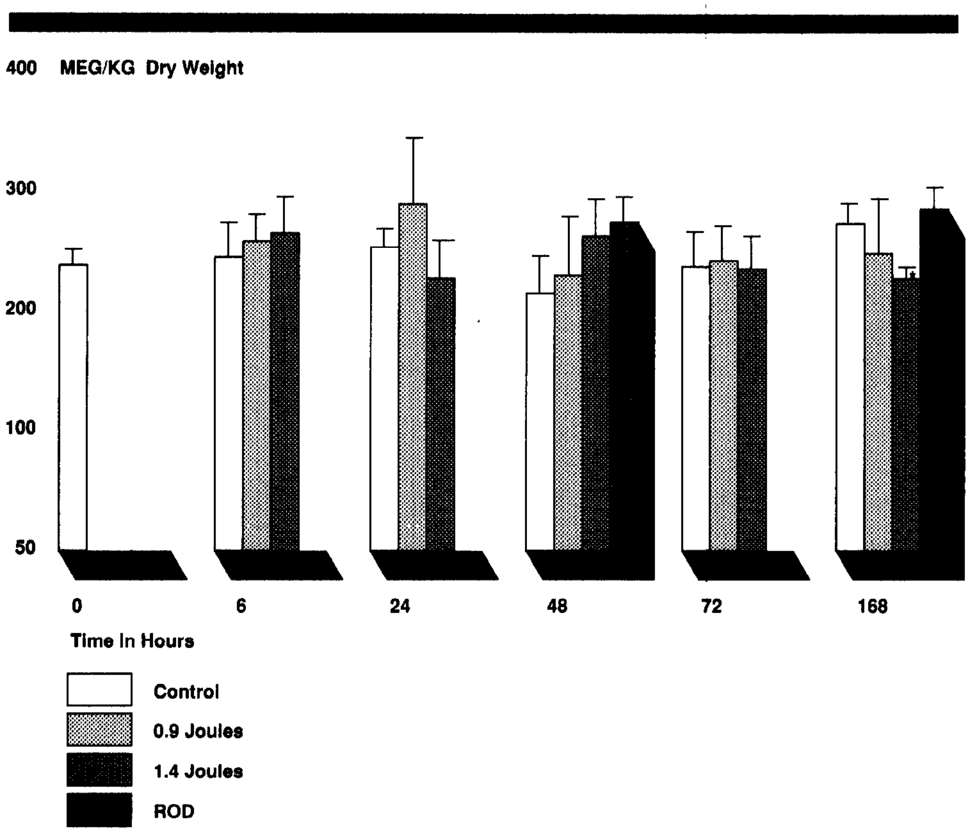


Means ±5.0. ** P<0.01: *P<0.05 of Control. + P<0.05. 0.9 J v 1.43

Note: This figure indicates the amount of brain water in the white matter of the cerebral hemisphere for control cats and cats injured at different energy levels.

Source: LSU's final report on the first contract, p. 60.

Figure IX.3: Brain Potassium in the White Matter of the Cerebral Hemisphere for Control Cats and Cats Injured at Different Energy Levels



Means ±0.05. * P<0.05 of Control.

Note: This figure indicates the amount of brain potassium in the white matter of the cerebral hemisphere for control cats and cats injured at different energy levels.
Source: LSU's final report on the first contract, p. 62.

Schedule of Animals Used

On November 9, 1989, the principal investigator provided us the following schedule of animals used in the 10 main areas of the LSU research. This schedule accounts for the total number of cats (1) used, (2) not used, and (3) from which usable data was obtained. These 10 research areas include the 33 experiments outlined in June 1989 for GAO's medical review panel; together, these 10 areas comprise what the principal investigator describes as the main thrust of the work.

Table X.1: Animals Used in 10 Main Research Areas (Nov. 9, 1989)

	Number of cats
Brain catecholamines	
Total used	**57**
With usable data	37
Remainder:	20
Technique development	6
Assay check	4
Pilot study	3
Indeterminate	1
Technical failure	3
ICP outside acceptable range	3
Histology	
Total used	**25**
With usable data	19
Remainder:	6
Died after wounding	1
Poor fixation	5
Behavior	
Total used	**53**
With usable data	24
Remainder:	29
Died after wounding	19
Isoflurane deaths	6
Living but pregnant	3
Blind	1
Plasma catecholamines	
Total used	**37**
With usable data	32
Remainder:	5
ICPs outside acceptable pressure	4

(continued)

	Number of cats
Total:	**16**
Died postwounding	7
Respiratory problems	3
Massive brain bleeding	1
Inaccurate shot	1
Monitored only 4 of 6 hours	1
Splenectomized	3
Brain water and electrolytes	
Total used	**111**
With usable data	74
Remainder:	37
Died postwounding	19
Different anesthetic	8
Different trajectory	6
Massive brain hemorrhage	4
Miscellaneous	
Total used	**23**
With usable data	9
Remainder	14

The detailed DoD comments on the report findings and recommendations are provided in the enclosure. Additional technical changes have been separately provided. The Department appreciates the opportunity to comment on the draft report.

Sincerely,

Charles M Herzfeld

Charles M. Herzfeld

Enclosure

consistent with the mission of the Army Medical Research and Development Command to conduct medical research designed to support the soldier in the field.

- **FINDING B:** Evaluation Of The Research Under The Louisiana State University Project. The GAO reported that, to evaluate the scientific aspects of the project, the GAO convened a panel of medical experts to review the research and identify any areas warranting further investigation. The GAO explained that the panel of experts reviewed the contract proposals and various reports sent to the Army under the contract requirements--and provided both their individual comments and a summary compiled by the panel chairman. The GAO reported that most panelists expressed concerns about the research performance in some areas, with most concerned about management of the anesthesia and post-operative care. The GAO further reported, however, the panel concluded that the goals of the research are valid and that treatment of missile injury is important. According to the GAO, the panel strongly believes that progress in improving the outcome of brain injury can only be made through studies such as the Louisiana State University project and deemed the model unique and suitable for the investigations undertaken. The GAO also reported that, to the best of the panel's knowledge, no other group has developed such a model or studied and characterized it so extensively.

The GAO reported that the panel relied on the accreditation of Louisiana State University by the American Association for Accreditation of Laboratory Animal Care in concluding that care of the animals was adequate. In addition, the GAO noted that the panel was reassured by its chief consultant on the care of animals that Louisiana State University more than adequately met the Association's standards. According to the GAO, the panel did not believe it could judge the adequacy of the postoperative care procedures from the documentation reviewed. The GAO also reported, however, that the panel believed the anesthetics were adequate to protect the animals from pain during the wounding, commenting that the brain has no nerve endings per se and does not suffer pain postoperatively. In addition, the GAO reported that the panel concluded that the principal investigator is a highly respected member of the neuro-surgical community, with a long standing interest in missile injury and a unique clinical experience in the battlefield. The GAO reported that, while most panelists expressed concerns about research performance in some areas, the panel concluded that the project had merit and that funding for the project should continue. (p. 3, pp. 5-6, pp. 33-34, pp. 115-206/GAO Draft Report)

Now on pp. 3-4, 21, and 212-298.

Now on pp. 3-5, 21-26, and 41.

comparability in the depth of anesthesia among wounded and unwounded cats. The GAO concluded that questions about the control of general anesthesia used in the Louisiana State University project is one of several concerns that raise doubts about the validity of some of the research results. (pp. 3-4, pp. 6-7, pp. 34-41, pp. 70-71/GAO Draft Report)

DOD RESPONSE: Partially concur. The DoD agrees with the scientific panel that the anesthetics used were adequate to protect the animals from pain. However, the assertions of the veterinary anesthesiologists that the Louisiana State University studies lacked proper dose control of the anesthesia and, therefore, raised doubts about the validity of the research is a misstatement of the facts. The method of anesthesia control was completely proper and in no way invalidated the research data. The veterinary anesthesiologists expressed concern as to the need for maintenance of the animals at the same depth of anesthesia. The anesthesiologists advocated precise control of the dose and implied that, if the dose was not precisely controlled, it would be impossible to determine whether the pathophysiological changes were due solely to the injury or to a combination of the injury and the anesthesia. In fact, the only scientific method to determine precisely whether the anesthesia had any effect on the pathophysiological changes would be to produce brain trauma in an unanesthetized cat. Such an experiment would be totally unacceptable because it would be inhumane and it would violate animal welfare laws and regulations. The response to the same dose of anesthetic will vary significantly from subject to subject. This will occur even if serum or alveolar levels are measured and kept uniform. The key is not a standardized dose, but rather a standardized response. In the work at Louisiana State University, this was accomplished by evaluating both eyelid and toe pressure reflexes. The anesthetic agent was initially given intraperitoneally to avoid stress to the cat and injury to the animal handler. Additional doses of anesthesia were administered via the intravenous route so that a uniform response was maintained. It is important to note that any form of anesthesia will have, by definition, an effect on the central nervous system, to include cerebral blood flow, as well as other systemic effects. The small effect of pentobarbital anesthesia on cerebral blood flow has been accounted for by the principal investigator in his scientific design and evaluation of the data. It is to account for effects such as this that control groups are used in research. It should be pointed out that the scientific panel deemed pentobarbital an appropriate anesthetic for this study.

Initial intraperitoneal and follow up intravenous anesthetic doses were determined by the Louisiana State University group by conducting more that 50 meticulously deficits produced by the wounding and the effects of various treatment regimens to ameliorate those deficits. Thus, the details of the postoperative records should not raise any concerns about the validity of the research results.

- **FINDING E:** **Questions About Other Aspects Of Research Performance**. The GAO reported that the lack of detail in other aspects of the Louisiana State University research performance raised additional questions from the veterinary anesthesiologists about the validity of the reported results. The GAO observed, for example, that three anesthesiologists, who commented on the blood gas experiments, indicated that the reported data on oxygen and carbon dioxide levels suggest measurement errors and that the project researchers were unable to control blood gases. According to the GAO, the anesthesiologists believe that, unless the incongruities in the blood gas data are explained, the related research results may be invalidated.

The GAO also reported that questions were raised about the trauma model used in the project. According to the GAO, the Army awarded the contracts based on the assumption that a valid model existed for studying fragment injuries and testing various treatment drugs. The GAO reported, however, that two of the anesthesiologists found that the model does not predictably produce graded responses, while three of the anesthesiologists commented on the high failure rate of the model. In this regard, the GAO noted that the failure rate was more than two and one-half times greater than that estimated by Louisiana State University.

Finally, the GAO found that not all the data included in the research have been reported. The GAO found, for example, that the reported results do not discuss data from experimental failures. The GAO compared the laboratory notebooks with reports submitted to the Army and found substantial difference between the number of animals used and the number for which data were reported. According to the GAO, all five anesthesiologists believe that, on the basis of the current emphasis of minimizing the use of experimental animals for both humane and cost purposes, there is a marked disproportion between reported and unreported animals in the Louisiana State University project. The GAO concluded that questions about these other aspects raise doubts about the validity of some of the research results. The GAO further concluded that the concerns discussed in Findings C, D, and E, taken together, suggest the need for a careful reassessment of the project's future. (pp. 3-4, p. 8, pp. 35-36, pp. 46-55, pp. 70-71/GAO Draft Report)

Now on pp. 3-6, 22, 28-33, and 41-42.

the law. The GAO noted that similar language has been included in DoD Appropriation Acts since FY 1984. The GAO observed that, while the legislative history shows congressional concern regarding the use of cats and dogs, it does not indicate why the law prohibits their use for training purposes, but not for other purposes. The GAO further concluded that, because it involves research and does not involve training, the Louisiana State University research does not violate the public law limiting the use of cats and dogs in DoD projects. (p. 4, pp. 56-57/GAO Draft Report)

Now on pp. 3-4 and 34.

DOD RESPONSE: Concur. Public Law 100-202 only prohibits DoD from purchasing or using dogs and cats for the training of DoD students or other personnel in surgical or other medical treatment of ballistic wounds. The Army contract with Louisiana State University is strictly for research and does not involve training. Therefore, it is in compliance with Public Law 100-202.

- **FINDING G: Army Monitoring Of Contract Performance.** The GAO reported that Army contract monitoring procedures, such as site visits and review of progress reports, provide the means for determining whether the research activities are consistent with contract requirements and ensure that the research results will be of value. In the case of research projects, the GAO reported that the Army appoints a technical person as the contracting officer representative to assist the contracting officer in monitoring contract performance. The GAO found, however, that although such an individual has a critical role in monitoring the technical aspects of research performance, four different contracting officer representatives have been appointed since 1983--and for a significant portion of the time, there has been no technical contracting officer representative.

The GAO also found that the monitoring by the contracting officer representative has been limited and lacked depth. The GAO cited several examples indicating that, even when there was one, the technical representative made infrequent site visits to the project. In addition, the GAO pointed out that the reports prepared on those visits do not indicate any follow-up of concerns noted in the reports.

The GAO further found that the majority of the required contract reports have been submitted to the Army late and there is little evidence that the Army has attempted to enforce its reporting provisions. The GAO also found that the Louisiana State University did not make the changes to the research scope and methodology recommended by the Army peer review panel evaluating the second contract proposal. The GAO reported that the Army took no action to assure these recommendations were implemented after the concerns were communicated to Louisiana State University. Finally, for the current contract. In addition to the documented visits, numerous undocumented telephone conversations took place between the contracting officer's representative and the principal investigator at Louisiana State University to discuss progress of the study. (Steps have been taken to make sure future telephone contacts are fully documented.) More frequent visits to such a fully accredited institution were not deemed necessary. It is correct that reporting dates were not met. The contracting officer's representative and the contract specialist did remind the principal investigator on many occasions that the required Army reporting dates were not being met, but the Army made no other attempt to enforce the reporting deadlines. An enforcement mechanism is now in place that requires the contract specialist to return all vouchers unpaid to the contractor if required reports have not been submitted on time.

Concerning the Peer Review Panel comments, Louisiana State University did not respond to those comments because they were intended only for internal Army review to determine and rank the scientific merit of all proposals submitted for possible funding. The Peer Review Panel's comments were not intended to be transmitted to the contractor. Instead, they were forwarded to an After-Action Committee that made the final determination on what was included in the contract with Louisiana State University, based on Army requirements. The After-Action Committee, with the concurrence of the acquisition management liaison officer, voted to accept the proposal "as is." Thus, the peer review comments were not sent to Louisiana State University for comment or incorporation into the proposal.

The changes made by Louisiana State University did not constitute changes of methodologies, stated objectives of research effort, or of the phenomenon or phenomena under study. We do agree, however, that the changes should have been discussed with the Army before implementation. The research investigators have been advised to follow such pre-consultation/pre-approval procedures before making any changes in the future.

- **FINDING H: Technical Assistance Provided By The Army**. The GAO observed that contract monitoring procedures give the Army the opportunity to (1) provide technical assistance, (2) guide and direct aspects of the research, (3) participate in decision making, and, (4) thereby, increase the probability of success. The GAO found that the Army only provided technical assistance early in the research effort, when the researchers experienced difficulty with the gun. The GAO concluded, however, that assistance was not provided at other times when it appeared to be appropriate to direct or participate in decision making to help resolve performance related issues.

brain wounds. The change in compounds was not a change in the methodologies. It has, however, been discussed with the Army and the Army has documented its agreement with using the newly developed drugs.

* * * * *

RECOMMENDATIONS

- **RECOMMENDATION 1:** The GAO recommended that the Secretary of Defense decide if the benefits of the Louisiana State University project on brain wound research have already been substantially achieved--and if the Secretary of Defense determines that the benefits have been substantially achieved, the project should not continue. (p. 9, p. 72/GAO Draft Report)

Now on pp. 6 and 42.

DOD RESPONSE: Concur. During September 1990, the Office of the Director, Defense Research and Engineering, will conduct a review of pertinent reports and documents associated with the brain wound research project. The review will include the reports of the Army and Louisiana State University studies of the project conducted concurrent with the GAO study. Other pertinent documents and records will also be included. Each of the expressed concerns of the GAO will be considered in the process to determine the overall impact they have on the conduct of the research. The review will permit an assessment of the achievements to date, as well as those that remain to be accomplished. If the objectives have been substantially achieved, the Director, Defense Research and Engineering, will recommend to the Secretary that the project be terminated.

- **RECOMMENDATION 2:** The GAO recommended that, if the Secretary of Defense finds that the benefits of the research project have not been substantially achieved, the Secretary should review the concerns raised in the GAO report to determine if continuing the contract will produce additional useful information. (p.9, p. 72/GAO Draft Report)

Now on pp. 6 and 42.

DOD RESPONSE: Concur. If the review and assessment cited in the response to Recommendation 1 indicate that there are substantial additional benefits to be gained by continuing the project, each of the GAO concerns will be carefully evaluated to determine its effect on the project. Such an effort will be directed at determining whether any of them would prevent the project from producing additional substantial benefits. If all the concerns either are considered to have insignificant effects or can be corrected to eliminate any adverse effect, continuation of the project will be recommended.

Executive Summary

Purpose

After the Army made details public, in the summer of 1988, about a research project using cats to study shell and other fragment wounds to the brain, an intense debate began as to the project's usefulness. The subject of the debate was research being done under U.S. Army Medical Research and Development Command contracts with the Louisiana State University (LSU) School of Medicine in New Orleans. The Army defended the research as necessary to learn how to treat combat-incurred brain wounds more effectively so that soldiers could be returned to duty, thereby conserving military fighting strength. Critics of the research, including New Orleans-based animal welfare groups, argued that the project would not add to the body of knowledge already established by other research on the treatment of brain wounds.

At the request of Representative Robert L. Livingston, GAO reviewed the project to assess the likelihood that the remaining contract will provide useful results. GAO also reviewed the adequacy of the Army's management of the brain-wound research contracts with LSU and determined whether the research violates public law limiting the use of cats and dogs in Department of Defense (DOD) projects. The Defense Appropriations Act of 1991 (P.L. 101-511, Nov. 5, 1990) prohibits the Army from disbursing any of its fiscal year 1991 or prior years' appropriations to fund the LSU research, except for previously incurred costs, pending completion of GAO's review.

Background

The Army conducts a medical research and development program designed to support the soldier in the field and meet other Army health needs. The Army entered into two contracts, in succession, with LSU for brain-wound research. The performance period of the two contracts, the first of which began in 1983, has spanned 8 years. The total cost for the contracts will be about $2.1 million. As of November 9, 1989, about 700 cats had been used in the research.

GAO primarily used four sources of data: the Army, LSU, a panel of medical experts, and also veterinary anesthesiologists. To obtain information on the LSU contracts and how the Army managed them, GAO met with Army officials and reviewed contract files. GAO also visited the research laboratory at LSU several times and discussed the research with the principal investigator and LSU officials. To evaluate the usefulness of the project and identify any areas warranting further review, GAO convened a panel of experts, representing a variety of medical specialties, in June 1989. In a day-long meeting, the panel reviewed the contract proposals and various project reports LSU submitted to the Army under operating procedures for monitoring the performance of the research and did not provide technical assistance when appropriate. (See ch. 3.)

Principal Findings

Expert Medical Panel Believes LSU Project Has Merit

The GAO panel concluded that the goals of the research are valid. The treatment of missile injury, both on the battlefield and in civilian circumstances, is important. The panel strongly believes that progress in improving outcome of brain injury can only be made through studies such as this one. The model was deemed to be unique and suitable for the investigations undertaken. To the best of the panel's knowledge, no group other than LSU has developed such a model or studied and characterized it so extensively while pursuing therapeutic strategies aimed at improving outcome of brain injury.

The panel relied on the American Association for Accreditation of Laboratory Animal Care's[1] accreditation of LSU in concluding that care of the animals was adequate. Furthermore, the panel was assured, by its chief consultant on the care of animals, that LSU has more than adequately met the association's standards. The panel did not believe that it could judge the adequacy of the postoperative care procedures from the project documentation they reviewed. But the panel believes that the anesthetics were adequate to protect the animals from pain during the wounding itself. Further, the panel commented that the brain has no nerve endings per se and does not suffer pain postoperatively.

The panel also concluded that the principal investigator is a highly respected member of the neurosurgical community with a long-standing interest in missile injury and a unique clinical experience in the battlefield. Although, as mentioned earlier, the panel concluded that the project had merit, it expressed concerns about the performance of the research in some areas.

Questions About Control of General Anesthesia

To compare wounded animals with other wounded animals and to compare wounded animals with unwounded ones, animals should be maintained at the same depth of anesthesia. Some of the measurements

[1]This is an organization that accredits institutions engaged in animal research. Institutions voluntarily seek accreditation that, if obtained, must be periodically renewed.

- blood gas measurement values that were beyond the realm of possibility,[2]
- lack of different responses to injuries of increasing severity,
- the number of cats used in the research that did not result in usable data, and
- data from a large number of cats that were excluded from the reported results. (See ch. 2.)

Army's Management of the LSU Contracts Inadequate

The Army has not adequately monitored the technical performance of the LSU contracts so as to increase the probability of project success by (1) participating in project decision making and (2) identifying technical assistance needs. The contracting officer's representative—the primary individual responsible for monitoring contract technical performance—made infrequent site visits; frequently allowed contractually required progress reports to be submitted late, combined with other reports, or not submitted at all; and did not thoroughly review the reports that were submitted. (See ch. 3.)

Recommendation

GAO recommends first that the Secretary decide if the project's benefits already substantially have been achieved. If so, the Secretary should not continue the project.

If the Secretary finds that the benefits substantially have not been achieved, GAO recommends that he review the concerns raised in this report to determine if continuing the contract will produce additional useful information. If, after this review, the Secretary finds it desirable to continue the project, then GAO further recommends that he ensure that the concerns GAO identified have been resolved.

Agency Comments

DOD and LSU provided written comments on a draft of this report. DOD partially agreed with GAO's findings on the Army's management and monitoring of the LSU contract and has taken corrective actions. In addition, DOD concurred with GAO's recommendations on DOD procedures to decide whether to continue funding of the LSU project on brain-wound research. DOD has scheduled reviews and assessments of the brain-wound research to implement these recommendations.

[2]Blood gas concentrations are one measure of the depth of anesthesia.

Contents

BRIEF SUMMARY OF EXPERIME 3

03 Year- Regional BBB permeability in the cat quantified by PS Products of Aminoisobutyric Acid and Polyethylene Glycols (MW 400, 900)

- Effect of a missile wound upon the cat's BBB quantified by PS product changes

	MONTHS	CATS
Perfect Techniques	1	10
PS Products		
1) Aminoisobutyric Acid	2-5	40
2) Polyethylene Glycol MW400	6-9	40
3) Polyethylene Glycol MW 900	10-12	40
A) preliminary cats and cats required to obtain best sacrifice time		50
	TOTAL CATS	180

Problem Areas

1) Because of the current high cat usage at LSU Dr. Gonzales, head of animal care, can guarantee only 100 cats (6-7#, 6 months old) from Louisiana sources. These cats will cost $25.00-$45.00. 80-85 cats/year may have to come from commercial sources. Commercial cats will cost about $175.00 each including shipping charges. Dr. Gonzales states that he will make every attempt to supply additional cats (i.e., $>$ 100) from local sources. If he is successful the animal costs will drop dramatically. This will leave perhaps $5,000-$10,000 additional monies each year. This money will be spent doing additional experiments (See 2 and 4 below). Missile scaling problems preclude using smaller test animals. Cats are much more uniform test animals than are dogs.

2) Accurate PS determination - A recent, to be published communication from Dr. Ronald Blasberg (NIH) (Theoretical Analysis of Experimental Parameters which Influence the Determination of Reliable Transfer Constants Across the Blood Brain Barrier from Single Time Experiments, by Blasberg RG, Patlak CS, Fenstermacher JD; submitted to J Cereb Blood Flow Metabol) indicates that a true PS is difficult to obtain from a single time point measurement. Thus, we have included 50 extra cats during the 03 year to empirically determine the best sampling period for the 3 test molecules (i.e., 10, 20, 30 minutes)

3) PS for the 3 test molecules 03 year (AIBA, 400 MW PEG, 900 MW PEG) - Blasberg has determined AIBA PS in the rat, $0.9\text{-}2.0 \times 10^{-3}$ml min^{-1} gm^{-1}. To my knowledge PS for AIBA and PEG have not been established for the cat. We will do this in our control cats.

4) Vc determinations - The intravascular volume in which the test molecules are distributed during the experiment will be the plasma. In his paper Blasberg shows that tissue plasma volume and arterial plasma volume are

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Sample of our ongoing research -
Extraction and PS Experiments
as will be done in 03 yr

periment number: 10 2/2/82
periment Type: N20-O2 Anesthesia Type: N20-O2
ight of animal: 325 grams Hemocrit count: 0.00

me of BLOOD sample 0.1670
ckgrounds: Channel 1 16.40 Channel 2, 9.70

		Raw counts			-BLOOD DPM-		-BRAIN DPM-				
	Weight	CH1	CH2	AES	C14	H3	C14/gm	H3/gm	CBF	Ki	E
[illegible]		29850.7	6001.4	0.5475	21557.3	154709.					
70	0.0242	1631.7	461.5	0.6407	21557.3	154709.	50440.9	300834	0.7862	0.6534	0.8310
71	0.0272	2174.6	664.7	0.6477	21557.3	154709	63812.5	353265	0.9946	0.7672	0.7714
72	0.0509	4411.1	1200.4	0.6414	21557.3	154709	63070.4	389518	0.9832	0.8460	0.8604
73	0.0577	5320.5	1442.9	0.6422	21557.3	154709	66820.1	414411	1.0416	0.9000	0.8641
74	0.0296	1657.9	477.6	0.6464	21557.3	154709	42038.4	248133	0.6552	0.5389	0.8225
75	0.0755	4444.4	1183.1	0.6413	21557.3	154709	41920.2	264935	0.6534	0.5754	0.8806
76	0.0544	3747.4	976.9	0.6432	21557.3	154709	47702.8	309514	0.7435	0.6722	0.9041
77	1.0000	1.0	1.0	0.6505	21557.3	154709	-22.9	-65.0	-0.0004	-0.0001	0.3957
78	0.0431	3174.0	864.6	0.6437	21557.3	154709	53144.9	329694	0.8283	0.7160	0.8644
79	0.0444	3720.9	976.1	0.6512	21557.3	154709	57129.2	373072	0.8904	0.8102	0.9099
80	0.0332	1812.2	493.0	0.6385	21557.3	154709	39574.1	244898	0.6168	0.5319	0.8623
81	0.0374	2456.0	654.5	0.6433	21557.3	154709	46244.4	294049	0.7208	0.6386	0.8860
82	0.0326	2201.9	608.2	0.6379	21557.3	154709	49993.4	303437	0.7792	0.6590	0.8457
83	0.0274	1653.4	427.1	0.6417	21557.3	154709	41043.2	270300	0.6397	0.5870	0.9177
84	0.0143	596.4	172.3	0.6441	21557.3	154709	30431.8	182101	0.4743	0.3955	0.8338
85	0.0176	348.8	308.6	0.6335	21557.3	154709	46827.2	74665.9	0.7299	0.1622	0.2222
86	0.0285	1378.5	394.8	0.6421	21557.3	154709	36365.0	214945	0.5668	0.4668	0.8236
87	0.0414	3691.3	989.1	0.6437	21557.3	154709	63384.6	399795	0.9879	0.8683	0.8789
88	0.0697	3021.0	734.0	0.6411	21557.3	154709	28044.8	195702	0.4371	0.4250	0.9724
89	0.0289	2904.4	833.8	0.6480	21557.3	154709	75501.9	446328	1.1768	0.9693	0.8237
90	0.0423	1722.5	444.3	0.6416	21557.3	154709	27689.2	182508	0.4316	0.3964	0.9184
91	0.0561	5204.2	1439.7	0.6462	21557.3	154709	67826.1	414611	1.0572	0.9005	0.8518

NOTE E, last column converted to PS by

$$PS = -F^{*} \ln(1-E)$$

* F = CBF column

In these experiments Vc = 1.0

Carpenter Research Associates
P. O. BOX 193
EDGEWOOD, MARYLAND 21040

November 9, 1981

Michael E. Carey, M.D.
Professor
Department of Neurosurgery
LSU Medical Center
NewOrleans LA 70112

Good Morning Dr. Carey

Mr. Sturdivan has talked to me about your requirment for an Air Gun and velocity measuring system for very small spheres. I have done a lot of work in this field and I know that we could fabricate the equipment that you would need to do the job. The following is an informal quote for the job and reflects todays prices. I would not anticipate much higher costs in the near future.

1. Air Gun with Pressure Regulator and barrels for spheres from 3/64 inch to 1/8 inch. $2,000.00
2. Velocity measuring equipment for these small missiles $2,000.00
3. Deliver equipment to LSU, install, and instruct personnel in proper operation. (2 days on site) $1,500.00

The backstop for this gun could consist of several layers of ballistic cloth which we would furnish at no additional charge. The only thing that you would provide would be an operating site and a cylinder of high pressure Helium. We would expect that we could give you delivery within 60 days of receipt of a firm order.

Sincerely yours

Robert E. Carpenter

REC/ms

CURRICULUM VITAE

NAME:	Michael Emmett Carey
RESIDENCE:	[Deleted by GAO.]
PROFESSIONAL ADDRESS:	Department of Neurosurgery Louisiana State University School of Medicine 1542 Tulane Avenue New Orleans, Louisiana 70112
BORN:	[Deleted by GAO.]
MARITAL STATUS:	[Deleted by GAO.]
DEGREES:	A.B. - Yale College, New Haven, Connecticut, 1956 M.D. - Cornell University Medical College, N.Y.C., 1960 M.S. - (Neurosurgery) University of Minnesota, Minneapolis, 1970
INTERNSHIP:	University of Minnesota Hospitals, 1-7-60 to 30-6-61 (General Surgery)
RESIDENCY:	University of Minnesota Hospitals, 1-7-61 to 30-6-62 (General Surgery) University of Minnesota Hospitals 1-7-62 to 30-6-67 (Neurosurgery) Mayo Clinic, Rochester, Minn. 1-1-65 to 30-6-65 Rotation from University of Minnesota
LICENSURE:	Connecticut, Louisiana
SPECIALTY BOARDS:	American Board of Neurological Surgery, 1970
PRIVATE PRACTICE:	Hartford, Connecticut, 1967-1968
ARMY:	Commanding Officer, 378th Medical Detachment (KE) and Chief of Neurosurgery, 312th-91st Evacuation Hospitals, Chu Lai, Republic of Vietnam 1968-1969. Chief of Neurosurgery, William Beaumont General Hospital, El Paso, Texas, 1969-1970 Colonel, U.S. Army Reserve (MC) 1978 to present "A" designation.
FACULTY APPOINTMENTS:	Consultant of Neurosurgery University of Connecticut, 1967-1968 Assistant Professor of Surgery/Neurosurgery, Louisiana State Medical Center, 1970-1974. Associate Professor of Surgery/Neurosurgery, Louisiana State University Medical Center, 1974-1978. Professor of Neurosurgery, Louisiana State University Medical Center, 1978 to present

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PUBLICATIONS

War Neurosurgery

1. Carey, M.E., Young, H.F., Mathis, J.L., Forysthe, J.: A bacteriological study of craniocerebral missile wounds from Vietnam. J Neurosurg 34:145-154, 1971

2. Carey, M.E., Young, H.F., Mathis, J.L.: The bacterial contamination of indriven bone fragments associated with craniocerebral missile wounds in Vietnam. Mil Med 135:1161-1165, 1970

3. Carey, M.E., Young, H.F., Mathis, J.L.: The neurosurgical treatment of craniocerebral missile wounds in Vietnam. Surg Gynec Obstet 135:386-390, 1972.

4. Carey, M.E., Young, H.F., Mathis, J.L.: The neurosurgical treatment of craniocerebral missile wounds in Vietnam. An analysis of 224 Vietnamese sustaining brain wounds. The Vietnam Military Medical Journal 40:25-36, 1972

5. Carey, M.E., Young, H.F., Mathis, J.L.: The outcome of 89 Americans and 224 Vietnamese sustaining brain wounds in Vietnam. Mil Med 139:281-284, 1974

6. Carey, M.E., Young, H.F., Rish, B.L., Mathis, J.L.: Late mortality and morbidity observed in a group of 102 American soldiers with a brain wound operated upon in Vietnam. Neurology (Minn.) 24: , 1974

7. Carey, M.E., Young, H.F., Rish, B.L., Mathis, J.L.: A follow up study of 103 American soldiers who sustained a missile wound in Vietnam. J. Neurosurg 41:542-549, 1975

8. Invited comment on paper by: Rish, B.L., Caveness, W.F. Dillion, J.D., Kistler, J.P., et al. ; Analysis of Brain Abscess after Penetrating Craniocerebral Injuries in Vietnam Neurosurgery 9: 535-541, 1981

9. Carey, M.E., Sacco, W., Merkler, J.: Analysis of fatal and non fatal head wounds incurred during combat in Vietnam by U.S. Forces Acta Chir Scand 508: (Wound Ballistics Fourth International Symposium) 351-356, 1982

-5-

PUBLICATIONS

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1. *Carey, M.E., Vela, A.R.: The effect of arterial hypotension upon the rate of cerebrospinal fluid formation in dogs. J Neurosurg 41:350-355, 1974*

2. *Vela, A.R., Carey, M.E., Thompson, B.M.: Further data on the acute effect of intravenous steroids on canine CSF secretion and absorption. J Neurosurg 50:477-482, 1979*

3. *Roheim, P.S., Carey, M.E., Forte, T., Vega, G.L.: Apolipoproteins in human cerebrospinal fluid. Proc Nat Acad Sci 76:4646-4649, 1979*

4. *Carey, M.E., Davson, H., Bradbury, M.W.B.: The effect of acute hypoglycemia upon cerebrospinal fluid production, iodide clearance and brain electrolytes in the rabbit. J Neurosurg. 54:370-379, 1981*

5. Carey, M.E., Davson, H., Bradbury, M.W.B.: The effect of acute hypoglycemia upon cerebralspinal fluid production, iodide clearance and brain electrolytes in the rabbit (with preliminary observations on the penetration of insulin into CSF) in Cervos- Navarro J., Fritschka, E., (eds): Cerebral Microcirculation and Metabolism New York Raven Press 1981

6. *Davson, H., Hollingsworth, J.G., Carey, M.E., Fenstermacher, J.D.: Ventriculocisternal perfusion of twelve amino acids in the rabbit. J Neurobiol 12: 293-318, 1982*

-7-

Papers in Preparation (February 1982)

1. Carey, M.E., Young, H.F., Rish, B.L. Mathis, J.L.: Sequelae of brain wounding in Vietnam

2. Carey, M.E., Young, H.F., Rish, B.L., Mathis, J.L.: Seizures after brain wounding in Vietnam

3. Carey, M.E., Sacco, W., Sturdivan, L.: Autopsy and ballistics studies on men dying from a brain wound in Vietnam

4. Carey, M.E., Tutton, R.: Computer brain scans following a brain missile wound

5. Carey, M.E., Mortality associated with brain abscesses at Charity Hospital

Books in Preparation

War Neurosurgery (for Surgeon General, US Army)

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Talks Presented

1. *Bacteriology of War Wounds: Gary Wratten Symposium, 1970 Walter Reed Institute of Research, Washington, D.C.*

2. *Bacteriology of War Wounds: Congress of Neurological Surgeons, 1970.*

3. *Mortality and Morbidity Associated with Craniocerebral Missile Wounds in Vietnam, Gary Wratten Symposium, 1971.*

4. *Mortality and Morbidity Associated with Craniocerebral Missile Wounds in Vietnam, Southern Society of Clinical Surgeons, 1971.*

5. *Mortality and Morbidity Analysis of 91 American Soldiers with Intracerebral Wounds: Congress of Neurologic Surgeons, 1971.*

6. *Intermediate Follow Up on 89 American Soldiers Who Sustained Intracerebral Missile Wounds in Vietnam. Gary Wratten Surgical Symposium. Walter Reed General Hospital, Washington, D.C., 1972.*

7. *Intermediate Follow Up on 89 American Soldiers Who Sustained Intracerebral Missile Wounds in Vietnam. Congress of Neurological Surgeons, Post Convention Meeting, Colorado Springs, Colo., 1972.*

8. *The Effect of Hypovolemic Hypotension of Cerebrospinal Fluid Formation in the Dog. Association for Academic Surgery, New Orleans, La. 1972.*

9. *The Effect of Systemic Arterial Hypotension Upon the Rate of Cerebrospinal Fluid Production in Dogs. American Association of Neurological Surgeons, Los Angeles, California, April 1973.*

10. *Neurologic Disabilities in Brain Injured Soldiers: A Three Year Follow Up. American Academy of Aphasia. Albuquerque, New Mexico, October 1973.*

11. *Late Mortality and Morbidity Observed in a Group of 103 American Soldiers with a Brain Wound Operated Upon in Vietnam. Southern Neurosurgical Society, Key Biscayne, Fla., February, 1974.*

12. *The Influence of Several Levels of Hypovolemic Hypotension upon the Rate of CSF Formation in the Dog. American Association of Neurologic Surgeons, St. Louis, Missouri, April 1974.*

13. *Current Concepts in Cerebral Spinal Fluid Physiology. American Association of Neurological Surgeons, Miami, April 1975.*

14. *Head Trauma. American Association of Neurological Surgeons, San Francisco, California, April 1976.*

15. *Spinal Cord Injury and Pancreatitis. American Association of Neurological Surgeons, San Francisco, California, April 1976.*

Proposal II

The principal investigator submitted the second proposal to the Army Medical Research and Development Command in January 1985. This proposal resulted in a second contract, "Experimental Study on a Brain Missile Wound; Ascertaining Pathophysiology and Evaluating Treatments to Lower Mortality and Morbidity." This contract began on April 14, 1986, and was scheduled to end on September 29, 1991. The following is the complete second proposal, except for "Budget" and "Budget Justification", which were deleted by the Department of the Army. Personal information on the researchers was deleted by GAO.

SCHOOL OF
MEDICINE IN NEW ORLEANS
Louisiana State University
Medical Center
1542 Tulane Avenue
New Orleans, LA 70112-2822
Telephone: (504) 568-6120

Department of Neurosurgery

March 11, 1986

Mr. Al Plum
Contract Specialist
Department of the Army
U.S. Army Research Acquisition Activity
Fort Detrick, Frederick
Maryland 21701-5014

Dear Mr. Plum:

Thank you for discussing our upcoming contract RFP-DAMD17-85-R-0016. I accept and will abide by the budget modifications stipulated by the DHHS review, ACN: 06-67615. Our revised budget is for $1,681,773.00 for 5 years.

I will devote 12.5% to 15% of my time to the project. Dr. Sarna and our other to be hired PhD will devote 100% of their time to the project.

Sincerely,

Michael E. Carey

Michael E. Carey, M.D.
Professor of Neurosurgery

MEC:eah

School of Allied Health Professions
School of Dentistry
School of Graduate Studies
School of Medicine in New Orleans
School of Medicine in Shreveport
School of Nursing

SUMMARY

Head wounds continue to be the most lethal battle wound accounting for almost half of all combat deaths. Acute neurosurgical mortality of brain wounds was 10-12% in WWII, Korea and also Vietnam. No advances in neurosurgical techniques have occurred in the last 10 years which would be expected to reduce head wound mortality further. One need not be fatalistic about brain wounds, however. In WWII and Korea one third of men so wounded returned to Army duty. Further reduction in mortality and morbidity requires a detailed understanding of the pathophysiology of brain missile wounds.

We have developed an experimental model of brain wounding in the cat using a 30 mg steel sphere fired through the skull into the brain. This model gives a graded response to study acute, subacute and long term physiologic changes. Transient apnea occurs frequently in our model and this is often reversible with respiratory support. This has direct relevance to the combat situation. Hemorrhage and its consequences continue to be an important cause of combat mortality. Our studies will include cats that remain normotensive after wounding and those that sustain one hour of severe hypotension after being wounded, simulating multiply injured soldiers.

We will use the most current physiological techniques to study the interrelationships of brain energy metabolites, neurotransmitters, cerebral blood flow, blood-brain barrier integrity and the behavior of the surviving cats. For drug evaluation we will score the cats' neurological and behavioral status both acutely and up to 21 days after wounding.

OUR PROJECT IS DESIGNED TO STUDY BOTH NEUROLOGICAL AND PATHOPHYSIOLOGICAL BASIS OF THE DEFICITS OBSERVED FOLLOWING MISSILE WOUNDING. THIS SHOULD QUICKLY LEAD TO SOUND PHYSIOLOGIC AND PHARMACOLOGIC METHODS TO AMELIORATE BRAIN DAMAGE CAUSED BY MISSILES.

issile wounds took place for US Army soldiers in the 25 years from WWII to Vietnam despite optimum vacuation and use of well-established neurosurgical techniques. In Vietnam, adjunctive therapy was eadily available (antibiotics, bountiful blood replacement, steroids, hyperosmotic agents and respiratory ipport) yet mortality from missile wounds of the brain did not improve compared to the 1944-1945 era hen antibiotics were first employed. An acute need exists, therefore, for detailed studies on the patho- ysiological effects of a missile wound to the brain in an experimental animal model. Then, more effec- ve adjunctive medical therapy can be developed that is specifically designed to sustain and improve brain inction following a brain wound. This approach offers the best chance to further reduce mortality and orbidity associated with a combat-incurred brain wound.

XPERIMENTAL BACKGROUND

Under the auspices of Army contract DAMD-17-83-C-3145, we have had about 8 months (19 January 5 October, 1984) to develop a laboratory model to study the effect of an experimental missile wound to ie brain of a cat. (Our experiments were stopped by DOD directives from September 1983 to January 984 and from 5 October 1984 to 14 January 1985.) Despite these restrictions, we have made significant ogress and are well on our way to fulfilling our 01 year contract goals. These include the following:

1. We have developed and established a laboratory instrument which can be used to create, for study, any types of wounds in laboratory animals. After significant modifications, the helium gun precisely fires 30mg steel sphere at varying velocities, (Fig. 2) which produces a reproducible wound simulating a frag- ent. In our laboratory model the sphere enters the cat's skull through the intact frontal bone and deposits s residual energy in the brain. We have chosen a frontal-occipital trajectory in the right hemisphere. ie missile perforates the frontal cortex, passes subcortically in the parietal area and ends in the occipital be (Figs. 3, 4, 7). The track is 1 to 2 centimeters from the brainstem. We have shown that a missile must ive a specific kinetic energy (KE) to penetrate the skull, 0.7 Joules (J) in our model. A non penetrating rike at this energy produces a local cerebral contusion, however, and this merits study because it may iuse a significant neurological deficit.[25]. Increasing missile KE above the threshold results in skull and ain penetration and deposits increasing energy within the brain. At low energies, brain damage

4. Following wounding we have observed hypertension, bradycardia, raised ICP (Fig. 10) and ıyperglycemia which is proportional to missile energy, Figs. 11, 12. The hyperglycemia presumably relects a stress-induced catecholamine response.[30-33]

5. We have observed recovery from a missile wound for 24 hours in 8 cats and for >60 days ı 2 cats wounded with sterile spheres. The 8 cats observed for 24 hours were purposely sacrificed and resumably would have lived as did the 2 long-term cats. One long-term cat was wounded at 0.93 J. he other at 1.35 J. Our model system clearly gives a graded response: The cat wounded with 1.35 J of nergy took longer to awaken than the 0.93 J animal and exhibited significant left hemiparesis for many ays. At 30 days, the 0.93 J cat exhibited only a left field cut, whereas the 1.35 J cat demonstrated a ›ft field cut, difficulty in arising from his right side, plus a tendency to circle right. Gerbils with erebral hemisphere ischemia also circle, probably related to basal ganglion neurotransmitter defiiency[34-35]. As with wounded humans,[25,36] both cats exhibited neurologic improvement with time. By 0 days both cats showed only left field cuts.

As perceived earlier by neurosurgeons[37] and neuropsychologists,[38] missile injury shows "striking ifferences from closed head injury"[37] in that focal brain damage is added to possible general (braintem) effects. Our model system appears to simulate the unique features of a human brain wound with reat fidelity and, indeed, the occurrence of distant damage away from the missile is a prominent nding.

Surprisingly few experiments on brain missile wounds have been done [39-51]. The most recent 4-51 involved chimpanzees wounded through a trephine opening in the skull. These interesting studies ocused very little on brain physiology per se. Furthermore, an unrealistically large missile was used 310 mg.). In Vietnam, the weight of the average fragment causing a brain wound was 110 mg[52]. Woundıg the brain through a trephine opening was unphysiologic and negated the effect of increased presures caused by the missile penetrating the closed skull. Perhaps this is why apnea was not a prominent

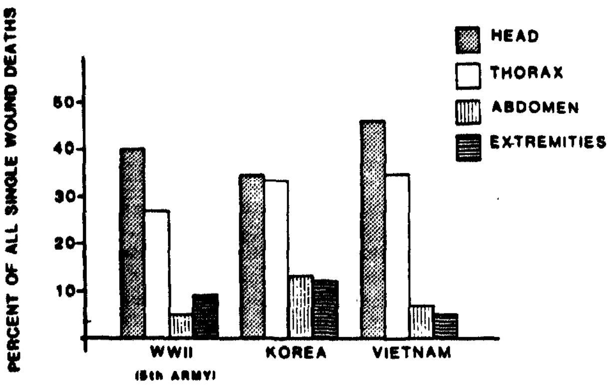
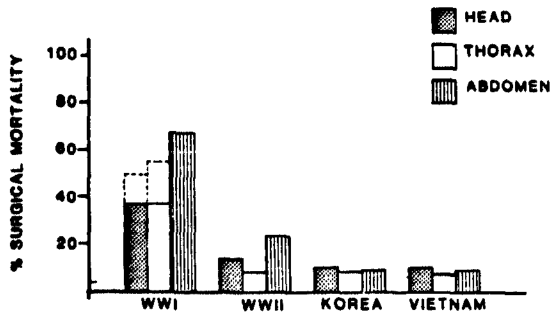


Fig.1:(left & below) Head wounds continue to be the most lethal combat wound.[1,3,4] No change in combat neurosurgical mortality has occurred since WWII.[6,21,22]



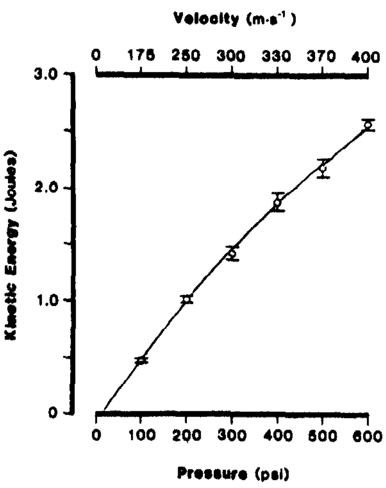


Fig.2: Pressure and velocity versus K.E. determinations in modified helium gun.

Fig.6: Coronal section with missile track. WWII casualty.[27]

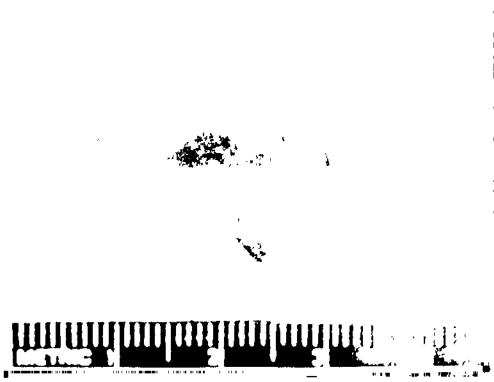


Fig.7: Sagittal section from our cat model showing missile track. Frontal entry site to the left.

Fig.8: Petechial hemorrhages in cerebral cortex adjacent to a missile wound. WWII case.[27]

Fig.9: Petechial hemorrhages about missile track in one of our experimental cats. High magnification.

HYPOTHESES

(1) A brain missile wound results in neurological and behavioral deficits which are directly related to the energy of deposit.

(2) An animal that is subject to hemorrhagic hypotension (MABP reduced to 40 mm Hg for 1 hour) following a brain missile wound is a valid simulation of the hypovolemic shock observed in many brain wounded soldiers.

(3) The fatal wound energy threshold is lower in animals subject to missile wound and hemorrhagic hypotension.

(4) Studies of the pathophysiologic status and the neurologic status of missile wounded animals are best studied concurrently to obtain sound physiologic and pharmacologic methods to ameliorate the effects of brain damage.

(5) Treatment(s) proposed can be effective in decreasing mortality and morbidity following brain missile wounding.

(6) A number of physiologic functions may serve as indicators of the severity of wounding. The glucose response is mediated by a massive sympathetic discharge and may be detrimental. The acute hypertension observed following high energy wounding may further increase brain damage by causing a breakdown of the blood-brain barrier and allow the entry of substrates into brain that would normally not penetrate.

(7) Brain missile wounding is associated with marked changes in cerebral energy metabolism, cerebral blood flow and neurotransmitter systems. These changes will be both focal and distal to the main missile track in the brain.

(8) Repetitive monitoring of physiologic and neurochemical functions in animals up to 21 days post-wounding will allow more direct correlations between these functions and the neurological deficits observed.

PLAN OF INVESTIGATION

BRAIN MISSILE INJURY

(A) NORMOTENSIVE ANIMALS
(B) HYPOTENSIVE ANIMALS
(hemorrhaged after injury
to effect MABP of 40 mmHg)

To be studied **concurrently**

INTERRELATIONS

I. NEUROLOGICAL STATUS

(a) Evaluation of mortality and morbidity

(b) Effect of various treatments on mortality and morbidity

(c) The pathophysiological consequences of the most efficacious treatments will be studied:
See IIa, IIb, IIc

II. PATHOPHYSIOLOGICAL STUDIES

(a) Acute physiological consequences (6 hours)

(b) Cerebral trauma: effects on

(i) Regional cerebral energy metabolism

(ii) Neuro transmitter systems

(iii) Cerebral blood flow

(iv) Integrity of the blood-brain barrier

(c) Repetitive monitoring of physiological and neurochemical consequences (including EEG and behavior) in animals kept alive for 21 days.

warmed blood, stored in acid-citrate-dextrose primed reservoir, will be reinfused one hour post wounding simulate resuscitation.

EVALUATION OF MORTALITY AND MORBIDITY

Rationale Our initial experiments will consist of wounding 2 series of untreated cats at several energies. We will ascertain 21 day post wounding behavior in all surviving cats and correlate post wounding behavic with wound energy. We will also discover whether hemorrhagic hypotension, (MABP 40mmHg, 1 hour) immediately after a brain wound will be associated with a lower $LD_{50}J$ wounding energy. We hypothesize that it will. The following experiments will be performed:

	Wound Energy (J)				Wound Energy (J)
Normotensive	0.7	0.93	1.35	Hypotensive	0.7-----1.35

We have ascertained that a wound energy of 1.35J kills about 50% of cats, i.e. is the $LD_{50}J$ wound energy. Surviving animals wounded at 1.35J have significant post-wounding neurologic deficits. Neurologic recovery scores of untreated cats wounded at 1.35J will be crucial and will form the baseline recovery data to which wounded and treated cats who survive for 21 days will be compared. Comparisons of wounded-untreated and wounded-treated 21 day recovery scores will largely delineate the efficacy of some drugs and treatment.

Methods Sixteen cats will be studied at each wound energy. Animals will be prepared and wounded in the usual fashion except that surgery will be sterile and we will use a sterile pellet for wounding. After wounding, we will monitor the animal 4 hours. Then, intravascular catheters will be withdrawn, incisions closed, penicillin given (50,000/kg IM), and the animal allowed to awaken. We will give nursing care and fluids, normal saline, prn I.P.

Data Analysis

1) Apneic response - Animals are "apneic" if they require respiratory support anytime after wounding. The number of apneic animals at each energy will be scored. LD_{50} wound energy = energy at which 8/16 animals require a respirator. Animals resuming voluntary respirations within 4 hours will be allowed to survive; those that do not will be perfused-fixed for brain histologic examination.

[2] Question of barbiturates interfering with evaluation of results because of possible cerebral protection.

[3] Could affect results.

[4] [No comment]

[5] Possibly. Pentobarbital and other [thought unfinished].

[6] Overall, anesthetic considerations were rather sloppy. Although for the most part, they provided adequate analgesic, their use could have affected the data. There is some question about the impact of barbiturates on data. Initially, PI states he won't use it, then does.

Phencyclidine has a wide variety of pharmacologic effects which would complicate the data.

[7] Yes, barbiturates have a "protective" effect on the brain and in some institutions and in the clinical setting are used as treatment for head injury (in the control of ICP). Therefore, barbiturates are not an ideal anesthetic for this project. I am also concerned about the number of times the anesthetic technique was changed. This does not make comparisons easy!

[8] Real concern about the variety of anesthetics.

Fourth Question: If you were assessing the research at the time it was proposed, overall, how would you rate the proposed anesthetic controls for this research for their capability to protect the animals from pain?

	5 Very high	4 Somewhat high	3 Neither low nor high	2 Somewhat low	1 Very low
1.			X		
2.	X				
3.			X		
4.		X			
5.					
6.				X	
7.					
8.			X		

[7] While I defer to others on the panel with expertise in animal medicine, I have grave concerns as to whether or not the animals, especially in the postop[erative care] period, experienced pain to their head wounds, raised ICP (which does cause headaches—ask anyone with pseudotumor cerebri [a syndrome of increased ICP associated with normal or small cerebral ventricles]) and [pain in] their wound sites for catheter placements.

[8] [No comment]

Second Question: In the research completed to date, have adequate medications been used to protect the animals from pain during the post-operative recovery period?

[1] [No comment]

[2] [No comment]

[3] Question was any requested—apparently not.

[4] [Respondent referenced his or her next answer.] I believe the anesthetic regimen(s) is unclear, as is the post-op analgesia and post-op care aspects of this proposal. Why use three different anesthetics? How will these alter data interpretation? How can one study using one anesthetic be compared to another using a different anesthetic? Even though this is probably not a painful procedure, it is important for the PI to indicate how he will monitor for pain, and what will he do if pain is apparent. Which analgesia will be utilized? When and under which conditions will the animals be euthanized because of pain? These questions need to be dealt with in the body of the text.

[5] Given expectations from human cases, pain would have been a minor problem. Cannot evaluate how pain problems were handled from material provided.

[6] [Respondent referenced his or her previous answer.] The postoperative management of chronic animals was unclear with respect to provision for animals' pain postoperatively. If analgesic would interfere with science and could not be provided, a justification to that effect would have been helpful. Furthermore, some endpoint should have been provided so as not to withhold analgesics indefinitely if they were indicated.

of ICP). Therefore, barbiturates are not an ideal anesthetic for this project. I am also concerned about the number of times the anesthetic technique was changed. This does not make comparisons easy!

[8] Anesthetics are a problem: (1) variables and (2) unknown effect of anesthetics.

[8] [No comment]

V. Investigators/ Equipment

A. Research as Proposed

First Question: Based on the curricula vitae contained in the proposals, did the research team have the qualifications needed to do the research as proposed?

[1] Although the qualifications of all involved appear adequate to conduct the proposed research, there is one concern regarding the overall low productivity of the group. Recent manuscripts sent to the Journal of Neurosurgery are viewed as encouraging.

[2] Limited publications in this field prior to beginning the research. Since the research has begun, the output of papers remains very low.

[3] Yes. Productivity of all researchers in past few years has been limited.

[4] This group has the qualifications to perform this research. However, I am concerned about the relatively poor productivity, especially of the PI. There are few publications from this group concerning this work, and I do not see that changing in the near future. I am pleased that he does have three papers in press now in Journal of Neurosurgery. This is helpful.

[5] In general, the team had good credentials but have not published as much as typically active investigators since this project began.

The persons doing the experiments (Sarna, Tortabi, Soblosky) [Sentence unfinished].

[6] Appears yes.

[7] This, to be sure, is a loaded question. As discussed at the meeting, it is interesting to note that the curricula vitae after the project started are more of a concern!

Third Question: If you were assessing the research at the time it was proposed, overall, how would you rate the qualifications of the research team to do the proposed research?

	5 Very high	4 Somewhat high	3 Neither low nor high	2 Somewhat low	1 Very low
1.			X		
2.		X			
3.		X			
4.			X		
5.			X		
6.		X			
7.				X	
8.		X			

[1] [No comment]

[2] [No comment]

[3] [No comment]

[4] [No comment]

[5] [No comment]

[6] [No comment]

[7] [No comment]

[8] [No comment]

the group. Recent manuscripts sent to the Journal of Neurosurgery are viewed as encouraging.

[2] Adequate, but not outstanding.

[3] Yes.

[4] [No comment]

[5] Yes.

[6] Yes. But all members' CVs who did work [were] not provided.

Investigators have not published much in recent years. CVs on Dr. McKowen and J. Bryan Farrell should have been included (or else a statement provided about their respective training for this project).

[7] [Respondent referenced his or her previous answer.] This, to be sure, is a loaded question. As discussed at the meeting, it is interesting to note that the curricula vitae after the project started are more of a concern!

[8] [No comment]

Second Question: Based on the information provided by the facilities and equipment display boards made available during the expert panel's meeting, did the research team have the facilities and equipment needed to do the research completed to date?

[1] [No comment]

[2] Yes.

[3] Yes.

[4] [No comment]

[5] Yes.

[6] Yes.

[7] [Respondent referenced his or her previous answer.] From the pictures and lists available, it appears that the facilities and equipment are adequate.

[5] The facilities appear adequate and appropriate to do most of the work. MR [magnetic resonance] is missing and would be a valuable addition.

[6] Yes.

[7] [Respondent referenced his or her previous answer.] From the pictures and lists available, it appears that the facilities and equipment are adequate.

[8] [No comment]

Fifth Question: Overall, how would you rate the research team's qualifications to do the research completed to date?

	5 Very high	4 Somewhat high	3 Neither low nor high	2 Somewhat low	1 Very low
1.			X		
2.		X			
3.		X			
4.			X		
5.		X			
6.		X			
7.			X		
8.		X			

[1] [No comment]

[2] [No comment]

[3] [No comment]

[4] Here, I am concerned with the lack of productivity of these investigators. However, my fears are somewhat alleviated by the fact that there are several, (3) manuscripts in press in Journal of Neurosurgery.

[5] [No comment]

[6] [No comment]

[7] [No comment]

Seventh Question: Overall, how would you rate the adequacy of the research team's facilities and equipment to do all of the research currently planned?

	5 Very high	4 Somewhat high	3 Neither low nor high	2 Somewhat low	1 Very low
1.		X			
2.		X			
3.	X				
4.	X				
5.		X			
6.	X				
7.		X			
8.	X				

[1] [No comment]

[2] [No comment]

[3] [No comment]

[4] [No comment]

[5] [No comment]

[6] [No comment]

[7] [No comment]

[8] [No comment]

[2] Is it necessary to have a separate model of missile injury? Cannot we learn as much from a closed head injury model since the brain has a limited means of response? It is probably not likely that pharmacologic treatment of GSW will differ from other types of head injury.

The low level of productivity is unsettling in reviewing this program. The recent submission of articles to the journals is encouraging.

The number of animals requested is not adequately justified. They strike this reviewer as too high a number for the experimental design. Statistical input would improve the program and the need for the animal numbers.

Trials of drugs should include some dose responses in order to establish specificity.

[3] [What were the] expectations of Army R[esearch] & D[evelopment] regarding project. Question open-ended or performance benchmarks. Question monitoring of contract.

Productivity of research group—less than stellar.

This is a very important project. Funding should continue. Productivity should be stimulated.

Question adequacy of peer review initially. Question relevant [thought not completed].

[4] I think this is an important area of research, and I believe work such as this needs to be done. However, I remain relatively unimpressed with this group of investigators, particularly in their publications and general productivity over the past years. Very few of their publications are directly applicable to the scientific aspects of this research. And there are only a few publications here at all in recent years. It is fair to say that up until now, this laboratory has not been a hotbed of activity and, although much data are presented, there are few publications so far to their credit.

[5] One issue of importance is the relative lack of published material by the research team since the project began. The reports of the work done on the project to date have been limited to presentations at meetings and

VII. Overall Assessment

First Question: Does the research, either as proposed or completed to date, provide new and valuable information about the treatment of penetrating brain wounds?

[1] The research completed to date does provide new information on penetrating brain wounds. Essentially, to date, an animal model has been developed and the pathophysiological sequelae of injury in this model have been described. The nature of the lesion and attendant changes in intracranial pressure and cerebral blood flow have been described. Also impaired vascular physiological reactivity and autoregulatory changes have been noted and, as such, have been linked to the injured-brain's increased vulnerability to secondary insult (hypotension). Overall, many of the above-described findings parallel observations made in other experimental situations considering brain injury in nonmissile injury. Thus, although the findings of this study are not revolutionary, they do provide data for an area which has not been previously investigated in comprehensive fashion. Additionally, information of this nature is essential for the development and study of the proposed treatment strategies.

[2] This cannot be answered based on data thus far because only the first phase of the program—the development of the model—has been done. Since this is the only model being utilized today, it may yield information on treatment.

To achieve these ends the level of productivity must be increased.

The hypotheses (p.19) [see app. II, hypotheses from second contract proposal, p. 121] do not define the goals for the specific treatment paradigms. The approach proposed is really based on the general ideas about closed head injury and ischemia and not specific to GSW. From review of material submitted, it is not clear how GSWs specifically might cause free radical changes, excitotoxicity. This would be appropriately part of the stated hypotheses.

[3] The lab is unique.

Research completed to date has made two valuable contributions: (1) developed and characterized a model for ballistic penetrating injury and (2) provided additional detail on the acute changes following injury, including apnea, autoregulation of CO_2, and hypoxemia responses. He has provided a more complete understanding of the ballistics and tissue responses.

[7] I do not feel that new information has come to light to date. The Crockard data has addressed many of these issues. Clinical papers by Becker et al. have addressed other of the issues.

The bottom line is, after reading the results of the experiments, has it changed my practice of neurosurgery in the case of gunshot wounds to the head, and the answer is "no." I do not believe it would change the practice of other neurosurgeons "on the front lines," and I must say that I seriously doubt that most neurosurgeons reading this report would respond any differently to this question than I have.

[8] Yes. I learned a great deal about penetrating injuries—lack of edema, low ICP unless there is hemorrhaging, loss of autoregulation. Penetrating injury is different from blunt trauma.

Second Question: Does the research, either as proposed or completed to date, duplicate existing research on this subject?

[1] [Respondent referenced his or her previous answer.] The research completed to date does provide new information on penetrating brain wounds. Essentially, to date, an animal model has been developed and the pathophysiological sequelae of injury in this model have been described. The nature of the lesion and attendant changes in intracranial pressure and cerebral blood flow have been described. Also impaired vascular physiological reactivity and autoregulatory changes have been noted and, as such, have been linked to the injured-brain's increased vulnerability to secondary insult (hypotension). Overall, many of the above-described findings parallel observations made in other experimental situations considering brain injury in nonmissile injury. Thus, although the findings of this study are not revolutionary, they do provide data for an area which has not been previously investigated in comprehensive fashion.

Additionally, information of this nature is essential for the development and study of the proposed treatment strategies.

This research does replicate some features previously explored in different models of experimental brain injury; however, in the field of penetrating brain wounds, this investigation must be considered unique. At present, no other group in the nation is conducting such studies and, as we are becoming an increasingly violent society with increased incidence of trauma to the brain, the need for such studies becomes all the more apparent.

the head and the answer is "no." I do not believe it would change the practice of other neurosurgeons "on the front lines," and I must say that I seriously doubt that most neurosurgeons reading this report would respond any differently to this question than I have.

[8] He has added considerable information to the Crockard study. The evolution of mechanical and ischemic injury—autoregulation.

Third Question: Does the research completed to date conform to the research proposals? If there have been changes, is the degree of change greater or less than that which might be expected to occur in research of this duration? Explain any significant changes and assess whether they enhanced or impaired the usefulness of the research in developing a treatment for penetrating brain wounds.

[1] In general, the research completed to date conforms to that originally proposed; yet there have been substantive changes in some of the experimental strategies. These changes are consistent with those seen in any developing research program and do not necessarily detract from the usefulness of the research. Given the format in which this reviewer noted these changes, some confusion did result and the logic of various approaches was initially unclear. However, after rereading the reports as well as that data provided at the meeting, the rationale and focus of these research projects became clear. Simply stated it appears that various metabolic aspects of the application have been abandoned, with greater emphasis on cerebral blood flow as determined through the use of microspheres. The approaches appear valid and are consistent with the long-range goal of developing effective treatments.

[2] This does not seem to be a critical issue. All research responds to the data acquired. The changes noted are appropriate and important to make maximum use of this model.

[3] There have been substantial changes in the research methodology. The changes have generally improved the project. Greater detail is provided in the research reports than in the methods proposed. Most of the methods in the original proposal were very superficial and brief. It seems that the projects have been carried out with adequate detail. Anesthesia variability and choice of anesthesia remain a potential problem.

[4] The research proposals and the work that has been completed do not necessarily perfectly match. This is to be expected with any research

Drug testing, while discussed in both protocols, to date (as seen in the information available for review) has not been done.

No publication of the results in a peer-reviewed journal has occurred to date, although we are told some data is soon to be published. It should be kept in mind that the project has been ongoing for 6 years, and one would think that some work would have already been published.

[8] The changes are expected and desirable.

Fourth Question: Does any of the research completed to date have immediate applicability to humans with penetrating brain wounds?

[1] In my opinion, little of the research completed to date has immediate applicability to humans with penetrating injuries. The widely noted finding of traumatically induced apnea does not appear particularly novel and, indeed, some caution should be exercised when translating these findings in cat[s] to humans sustaining brain wounds. The proposed drug treatment strategies offer the most promise for brain-wounded humans. However, until these drug studies are brought to closure, no comment can be made regarding their applicability to humans.

[2] Not as yet since no treatment trials have been completed. The potential is there but not yet realized.

[3] The research improves our understanding of what happens in the brain after ballistic injury. This greater understanding does not present an opportunity for a radical new approach, but does allow for more sensitive modulation of existing therapies for individual cases.

The current doctrine of Combat Casualty Care Course (C_4) could be changed to further emphasize the immediate airway management in head-injured battle casualties.

[4] I believe that much of the work that is to be done in the future has more applicability than that which has been done up until now. When the drugs are studied, they may lead to more applicability aspects. Up until now, the PI showed alterations in cerebral reactivity to perfusion pressure, CO_2, and O_2. This may be applicable from the point of view of hypotension, combined with the injury, so that it is clear that the person with a missile injury of the brain needs to have his blood pressure supported. There is also the idea of missile-induced apnea, which, although

characterizing autoregulation and the response of the GSW injured brain to CO_2 and O_2 variations.

The planned research on the effect of various drugs is important and may identify effects with application to certain ranges of injury.

[4] I believe there are several aspects of the research presented here which are new. One involves the autoregulatory characteristics of the cerebral circulation. The PI showed that the brain limited its ability to autoregulate. He also showed that the responsivity of the cerebral circulation to changes in pCO_2 and pO_2 is also limited following missile injury. Thus three aspects of cerebral physiology (autoregulation, CO_2, and O_2) were examined in this brain-injury model system. This information is new with this model. I would have preferred to see these physiological control mechanisms examined at several different times following injury rather than only at one time interval.

[5] The loss of autoregulatory blood flow and depressant effects of O_2 on brain blood flow with penetrating wounds of the brain are new or at least not mentioned in therapeutic discussions.

[6] (1) That brain missile wounding was associated with large increases in prostaglandin in CSF.

(2) Neurologic deficit in animal model did not correlate with presence of cerebral edema.

(3) Respiratory support immediately following brain wounding appears to be a major factor in survivability.

(4) Reperfusion following brain injury may be the wrong thing to do.

(5) Rising hematocrit following brain wounding.

(6) Missile wounding of brain can induce neurogenic pulmonary edema.

(7) Missile wounding precipitates local cerebral blood flow increase.

[7] This is a very difficult question to answer. There are no clinically relevant new data. However, since this is, to my knowledge, the only existing missile model in the cat, one must say that in that regard the results are "new" with respect to feline head injury.

Sixth Question (b): At the time the research was initially proposed, if you were on a peer review panel considering whether to fund the research, considering the information in the (1) researcher's final report on work completed under the first proposal and (2) second proposal, how likely would you have been to recommend that the second research proposal be funded?

	5 Very likely	4 Somewhat likely	3 Undecided	2 Somewhat unlikely	1 Highly unlikely
1.			X		
2.			X		
3.		X			
4.		X			
5.					
6.		X			
7.					X
8.	X				

[1] [No comment]

[2] [No comment]

[3] Very important to continue funding.

[4] There's some hesitation only because I prefer a proposal that is more mechanistic and detailed in nature, rather than a "shotgun"-type approach to an assortment of drug treatments. The hypotheses were not tightly outlined, and no mechanisms of action were specified.

[5] Between somewhat likely and undecided.

[6] [No comment]

[7] [No comment]

[8] [No comment]

for a contract to run from 1985 and 1991. Included also were the three annual reports covering July 1983 to June 1984, and April 14, 1986 to April 13, 1987 amd April 14, 1987 to April 13, 1988, and the final report of the first contract covering July 1983 to December 1985. The Chairman also received restricted material that had not been approved for public release, which consisted of an annual report dated April 1989 covering April 15, 1987 to April 14, 1988. In addition, diagrams of the research program were included, a new list of proposed treatment drugs to be used, and the curriculum vitae of Dr. Michael Carey, Dr. Gurcharan Singh Sarna, Dr. Dan Torbati, and Dr. Joseph Soblosky. In addition, we received abstracts and a paper to be published in the Journal of Neurosurgery.

Also in early June, a list of the questions that the GAO office felt might be relevant were sent to Dr. Jane. These research questions covered in general the research goals and the hypothesis, the experimental model to be used, animal care, anesthetic controls, the investigators, and the facilities.

The panel met on June 19, 1989, from 10:30 am to 6:00 pm. The questions that had been sent in early June were somewhat modified, but the list of questions that we were given was meant to serve as a guideline, but not to restrict the discussion in any way. The GAO had developed these questions for our use after review of the specific requirements under each contract and the expected items to be delivered under the contracts. GAO had also reviewed with Dr. Carey both contracts, the progress reports, the annual reports, and the final reports for each. They had, in addition, looked at the monitoring activities by the Army and the various medical research databases concerning the use of cats for brain/head wounds. They had discussions with the U.S. Army officials responsible for administering the two contracts. They also reviewed the criticism of Dr. Carey's work that had been made by animal rights groups. The panel felt that the questions formed a useful framework for discussing Dr. Carey's proposals.

Suitability of the experimental model for the research completed to date:

Rated 4.1.

Quality of the proposed care for animals to be used in the research:

Rated 3.75.

Quality of care given to the animals used in the research completed to date:

Rated 3.75.

Anesthetic controls for this research, as proposed, for their capability to protect the animals from pain:

Rated 3.0.

Anesthetic controls used in the research completed to date for their capability to protect the animals from pain:

Rated 4.0.

Qualifications of the research team to do the proposed research:

Rated 3.37.

Adequacy of the research team's facilities and equipment to do the proposed research:

Rated 4.25.

Research team's qualifications to do the research completed to date:

Rated 3.6.

Research team's qualifications to do all of the research currently planned:

Rated 3.6.

Research team's facilities and equipment to do the research completed to date:

Rated 4.5.

Adequacy of the research team's facilities and equipment to do all of the research currently planned:

Rated 4.37.

Considering only the information in the researcher's first proposal, how likely would you have been to recommend that the first research proposal be funded:

Rated 3.75.

2. Experimental Model

The model was deemed to be unique and suitable for the investigations that are being undertaken. To the best of the panel's knowledge, no other group has developed such a model or studied and characterized it so extensively while pursuing therapeutic strategies aimed at improving outcome of brain injury as is being done at LSU. On the other hand, one panel member said that it would be unusual for any two institutions to use the same model anyway and that in the 1970s at the University of Chicago, there was another model used by Crockard and Mullan.

3. Animal Care

The panel relied upon the AAALAC accreditation of LSU in order to conclude that care was adequate. See page 14(25-27) for additional comment.

Postoperative Care

Long-term postoperative care in the first proposal was not addressed because the cats were only to live for a six-hour period after the procedure. However, in the second proposal, the post-insult life of the cat was extended to 21 days. The panel recognized that adequate postoperative care is important and expressed concern that the protocol for postoperative care for the chronic surviving animals was not fully explicated. Based on the information provided, the panel could not judge the adequacy of the postoperative care procedures. Nonetheless, as assured by our chief consultant on the care of animals, he has more than adequately met the standards.

4. Anesthetic Controls

There was no concern that the animals might suffer during the wounding itself. Further the brain has no nerve endings per se and does not suffer pain postoperatively. The incisions are treated topically with lidocaine ointment for the adequate relief of pain. One reviewer commented that, although it is true that the brain has no pain fibers, that the skin, periosteum, and dura can appreciate pain

8

critical, and the discussion was open and frank and that their conclusion was that the work was well worthwhile.

Anesthetics: The panel expert on anesthetics felt that the following concerns voiced by the panel members were similar, but that, in his opinion, they did not obviate the overall importance of the proposal.

1. Anesthetics are a problem. variable, unknown effect of anesthetics
2. Question of barbiturates interfering with evaluation of results because of possible cerebral protection.
3. Because of the variation in anesthetics used some question must be raised as to how this effects results.
4. The variety of anesthetics and their possible interaction does raise several problems. Specifically, the use of multiple agents could complicate data analysis. This situation should require more consideration for more consistent forms of anesthesia use.
5. Animal management and anesthesia detail information is missing in the proposal.
6. It is unclear about which anesthetic in which dose was used in each animal. The present anesthetic protocol with cats (Torbati) with pentobarb. appears OK. However, within these protocol, brevital, pentobarb. and isoflurane are discussed as being utilized.
7. All anesthetics may effect outcome from neural injury. This is a difficult question; however, the PI should have dealt with this potential problem in the text of the proposal.
8. Anesthetic regime(s) is unclear as is the postop analgesic and postop care aspects of this proposal.
9. Anesthetic controls -- poor records, not explained, different drugs could affect results of the research.
10. Anesthesia variability and choices of anesthesia remain a potential problem.
11. Records for operative-postoperative care don't allow for evaluation. Anesthetic protocols were inconsistent and appear to differ. Postop management was questionable due to who monitored and when.
12. Anesthesia variability may interfere with the data.
13. Anesthetic controls poorly described.
14. Concern about the anesthetic controls and the type of anesthetics used. Barbiturates have a "protective" effect on the brain and could affect the results of the study. Also, concern about the number of times that the anesthetic was changed during the experiments.
15. Anesthetic controls or lack thereof could possibly affect research results.

GAO Note: See app. V for the detailed comments of each panel member.

Low Level of Productivity:

1. Low production.

2. The low level of productivity is unsettling in reviewing this program.

3. Lack of published data is disquieting.

4. There is some concern regarding the overall low productivity of the group, recent manuscripts sent to the Journal of Neurosurgery are viewed as encouraging.

5. Poor production of the group is a problem.

6. Productivity of the group was less than stellar.

7. No evaluation of drugs, and this was the main point of the study. No published data on study as of yet.

8. Overall concern is that the contract is not being followed and that the drug testing has not been started or completed. Also no publication of test results is a problem.

Study Results:

1. Apnea has been long noted in gunshot wounds.

2. It is probably not likely that pharmacologic treatment of GSW will differ from other types of head injury.

3. Observations on apnea are not new but certainly deserved, especially since they are not widely appreciated. One reviewer commented that one of the first observations on apnea contributing to morbidity and mortality in gunshot wounds of the head was made by Horsley in 1894 and published in Nature of that year. Currently, the advanced trauma life support courses emphasize protection of airway and intubation if necessary on all head injured victims. That reviewer, therefore, felt that the detrimental effect of apnea on patients was well known. The rest of the reviewers believe that old information is often rediscovered in new situations and in new ways and that this particular observation that bullet wounds to the head might be particularly prone to apnea was important and that "battlefield apnea" might well be of significances(and know

4. The likelihood of full neurological recovery seems remote.

5. Results of study to date -- interesting but not unique.

6. Although the findings of this study are not revolutionary, they do provide data for an area which has not been previously investigated in a comprehensive fashion.

7. It appears that various metabolic aspects of the application have been abandoned, with greater emphasis on CBF as determined through the use of microspheres. The approach appears valid and is consistent with the long range goal of developing effective treatments.

4. This is a valid model of brain (missile injury) which may occur on the battle field. No injury is perfect. This one is not either, but I believe it is the best kind of model to simulate battlefield injury available.

5. This research can be done only in animals. There are no other alternative techniques available which could give the same data. Absolutely not. The cat is an appropriate model for this study. There is much work in the literature already in cats and in other head injury models.

6. Insofar as any model testing animals is transferrable to humans, so is this one.

7. Very impressive facilities; he can do the experiments outlined. I have visited the laboratories and I must say the space and equipment resources are adequate to perform these studies. Essentially all of the equipment necessary to complete these studies is available on site.

8. I think this is an important area of research and I believe work such as this needs to be done. I believe this proposal does provide new and important information about the treatment of penetrating brain wounds. First, it has provided a model for future use that is a consistent graded model of missile injury. The earlier work done in this protocol does add to the body of literature in this area, i.e., edema is not an early problem, lack of all irregular CO_2 and O_2 responses of the cerebral circulation. The protocol still represents a promissory note-type study, since much or all of the work regarding use of pharmacological agents remains still to be done. It is likely that new information, positive and/or negative, regarding the usefulness of these agents will come from these experiments. I suppose that the major accomplishment to date is that the PI has developed a model of missile injury and is now ready to use the model for a variety of treatment modalities. (This is essentially the only laboratory in the world working in this area). While there has been some previous work done in this area over the years, no other laboratory is active at this time. Thus, the work does not represent duplication of previous work in the area and, basically, there is no competition with other laboratories at this time.

24. The PI has developed a working model from which all contemporary treatment strategies can be tested.

25. As LSU is an AAALAC accredited facility, I feel confident that the animals were well cared for. In a telephone conversation I had with the Chairman of their Animal of Care and Use Committee, I was impressed by his candor on this subject. He told me that Dr. Carey went out of his way to explain his research and answer all concerns of the Animal Care and Use Committee members. The Committee was quite comfortable, in his words, with all of his animal care and use activities.

26. Due to the fact that LSU is an AAALAC accredited facility, I assume that the veterinarian support staff are adequately trained to promote care.

27. LSU has an assurance on file with the NIH Office for the Protection for Research Risks (OPRR) and is both AAALAC accredited and in good standing. This assurance states that the attending veterinarian oversees animal care and use. I, therefore, assume ancillary personnel are adequately trained.

28. The essential point is that this research has developed a model for penetrating head wounds of the brain.

29. The work has led to better understanding of the dynamics of the penetrating brain wound and possible ways to enhance therapy.

30. Its greatest potential for treatment improvement is the means it gives to systematically evaluate any existing and proposed therapeutic action.

31. The hypotheses are broadly stated and do not fall into a traditional NIH format; however, they do appear consistent with the state-of-the-art of this particular field.

32. The choice of all therapeutic strategies appears based on contemporary thought.

33. The qualifications of all involved appear adequate to conduct the proposed research.

34. The research completed to date does provide new information on penetrating brain wounds. Essentially, to date, an animal model has been developed in the pathophysiological sequelae of injury in this model have been described.

Veterinary Anesthesiologists GAO Consulted

We consulted the following board-certified veterinary anesthesiologists, who are currently involved in research at state universities:

Dr. Richard M. Bednarski, DVM, MS
Assistant Professor of Anesthesiology
College of Veterinary Medicine
Ohio State University
Columbus, Ohio

Dr. Steve C. Haskins, DVM, MS
Professor of Anesthesiology and Intensive Care
School of Veterinary Medicine
University of California
Davis, California

Dr. Donald C. Sawyer, DVM, MS
Professor of Anesthesia
Adjunct Professor of Pharmacology and Toxicology
College of Veterinary Medicine
Michigan State University
East Lansing, Michigan

Dr. Lawrence R. Soma, VMD
Professor of Anesthesia and Clinical Pharmacology
School of Veterinary Medicine
University of Pennsylvania
Kennett Square, Pennsylvania

Dr. Cynthia M. Trim, MRCVS, DACVA, BVSc
Professor of Anesthesiology
College of Veterinary Medicine
University of Georgia
Joint Appointment: Department of Physiology and Pharmacology
Athens, Georgia

Ischemia and Pentobarbital

Saunders, M.L., and others. "The Effects of Graded Experimental Trauma on Cerebral Blood Flow and Responsiveness to CO_2." Journal of Neurosurgery, Vol. 51 (July 1979), pp. 18-26. [Anesthesia maintained with nitrous oxide.]

Jennett, S., L.H. Pitts, and J.B. North. "Rapid Cerebral Vasodilatation in Brief Hypoxia in Anaesthetized Animals." Quarterly Journal of Experimental Physiology, Vol. 66 (1981), pp. 447-63.

Shapiro, H.M. "Intracranial Hypertension: Therapeutic and Anesthetic Consideration." Anesthesiology, Vol. 43 (Oct. 1975), pp. 445-71. [This article is about humans.]

Weber, M. Furuse, M. Brock, and H. Dietz. "The Single Dye Passage. A New Technique for the Study of Cerebral Blood Flow Distribution." Stroke (Mar.-Apr. 1974), pp. 247-51.

Zee, C.M., and K. Shapiro. "The Origin of CSF Pulse Waves." In Intracranial Pressure VII, J.T. Hoff and A.L. Betz, eds. Berlin, Heidelberg: Springer-Verlag, 1989, pp. 164-65.

Kontos, H.A., and others. "Responses of Cerebral Arteries and Arterioles to Acute Hypotension and Hypertension." American Journal of Physiology. Vol. 234 (1978), pp. H371-83, or American Journal of Physiology: Heart Circ. Physiology. Vol. 3 (1978), pp. H371-H383.

Wei, E.P., and H.A. Kontos. "Responses of Cerebral Arterioles to Increased Venous Pressure." American Journal of Physiology, Vol. 243 (1982), pp. H442-H447, or American Journal of Physiology: Heart Circ. Physiology, Vol. 12 (1982), pp. H442-47.

Gyulai, L., and others. "Simultaneous ^{31}P- and ^{1}H-Nuclear Magnetic Resonance Studies of Hypoxia and Ischemia in the Cat Brain". Journal of Cerebral Blood Flow and Metabolism, Vol. 7 (1987), pp. 543-51. [Anesthesia maintained with nitrous oxide.]

Brock, M. "Regional Cerebral Blood Flow (rCBF) Changes Following Local Brain Compression in the Cat." Scandinavian Journal of Laboratory and Clinical Investigation, Suppl. 102 (1968).

Tanaka, K., and others. "Regional Flow-Metabolism Couple Following Middle Cerebral Artery Occlusion in Cats." Journal of Cerebral Blood

Brain Injury and Other Barbiturates

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Experiment Data

Table IX.1: Research Data on Arterial Blood Gases

Wound energy	Cat no.	Prewounding Resp. rate	Prewounding pO_2	Prewounding pCO_2	Prewounding pH	1 minute postwounding Resp. rate	1 minute postwounding pO_2	1 minute postwounding pCO_2	1 minute postwounding pH
0.9J	219	18	100.2	37.8	7.38	0	121.8	26.4	7.35
0.9J	227	14	81.2	31.8	7.48	8	63.7	35.7	7.41
0.9J[a]	231	8	82.7	46.8	7.36	10	65.7	50.4	7.28
0.9J	233	12	82.6	42.0	7.32	0	59.8	39.7	7.34
0.9J	239	16	102.9	40.8	7.32	20	121.7	39.9	7.35
1.4J	225	20	101.6	29.9	7.43	0	59.4	41.4	7.37
1.4J	228	24	74.3	40.7	7.33	19	71.7	41.9	7.33
1.4J	234	8	109.8	38.0	7.36	0	39.3	46.9	7.26
1.4J	237	14	113.6	40.9	7.30	0	46.8	50.9	7.25
1.4J[a]	243	10	111.4	42.3	7.40	14	61.2	51.9	7.30
2.4J	220	12	60.8	32.7	7.40	12	47.1	31.5	7.36
2.4J	223	12	127.5	44.0	7.37	6	120.0	36.6	7.33
2.4J	236	13	91.5	43.5	7.30	8	51.5	48.7	7.27
2.4J	241	12	105.8	44.6	7.32	0	57.9	50.3	7.36
2.4J[a]	244	16	120.6	40.1	7.38	21	72.9	50.9	7.31

Note: J = joules.

[a]Animals exhibiting significant decreased arterial pO_2, hypercarbia, and decreased pH without "central" respiratory depression.

Source: LSU's final report on the first contract, p. 47, submitted to the Army, February 10, 1987.

Table IX.2: Research Data on Intracranial Pressure—Control Cats

Time (mins.)	C261	C262	C263	C267	C269	Means	Standard deviation
0	9.0	8.5	8	4.0	4.0	6.7	2.489980
1	9.5	9.0	6	3.5	4.0	6.4	2.770379
3	9.0	8.0	6	4.0	6.0	6.6	1.949359
5	9.0	8.0	6	3.5	7.0	6.7	2.109502
10	9.0	8.5	6	4.0	6.5	6.8	2.018663
20	8.0	9.0	5	4.0	6.5	6.5	2.061553
30	8.5	9.5	5	4.0	6.0	6.6	2.329163
60	8.0	12.5	5	4.5	6.0	7.2	3.251923
120	14.5	11.0	9	6.0	15.0	11.1	3.781534
180	12.5	11.5	26	4.5	16.0	14.1	7.853343
240	13.0	12.0	29	4.0	20.0	15.6	9.396808
300	12.5	12.0	27	3.0	33.0	17.5	12.206556
360	12.5	15.0	28	7.5	22.0	17.0	8.070006

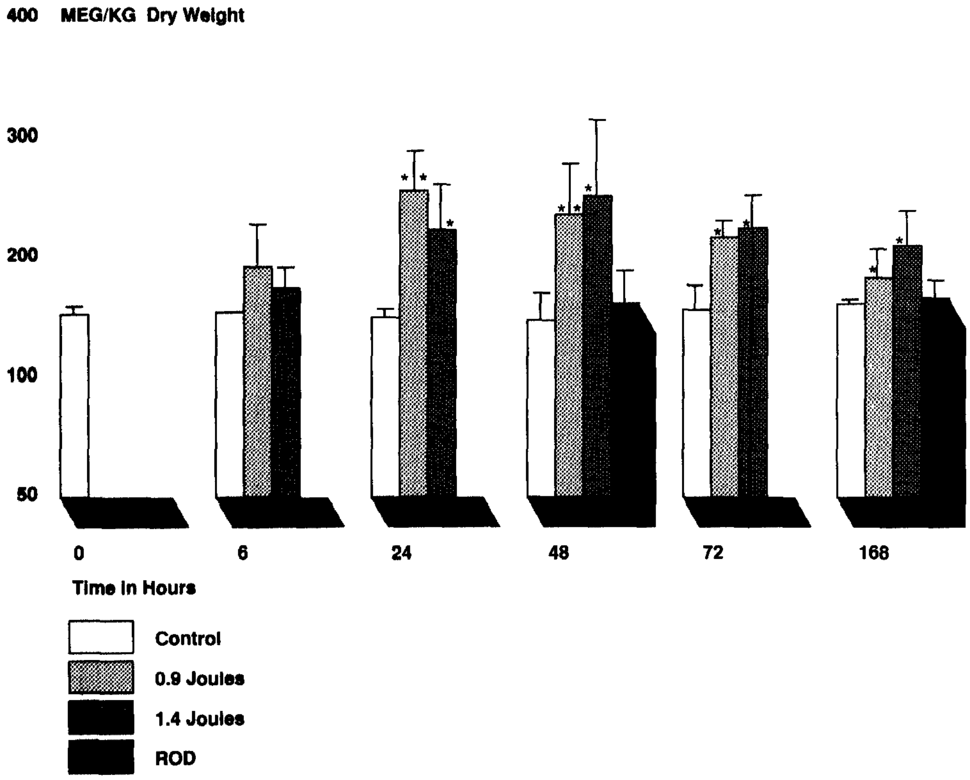
Source: LSU's final report on the first contract, p. 102.

Table IX.5: Research Data on Intracranial Pressure—Cats Wounded at 2.4 Joules

Time (mins.)	M220	M223	M236	M241	M244	Means	Standard deviation
0	5.3	5	3	7	12	6.46	3.407052
1	126.0	46	52	51	38	62.60	35.871995
3	98.0	72	40	73	29	62.40	27.790286
5	115.0	78	40	57	26	63.20	34.866890
10	60.0	67	48	49	24	49.60	16.349312
20	34.0	77	47	46	23	45.40	20.206435
30	33.0	82	35	41	23	42.80	22.851696
60	48.0	54	29	32	27	38.00	12.186058
120	55.0	46	48	36	32	43.30	9.316652
180	50.0	46	110	34	39	55.80	30.922484
240	58.0	46	19	28	30	36.20	15.594871
300	57.0	46	25	30	31	37.80	13.292855
360	60.0		43	21	43	41.75	15.986974

Source: LSU's final report on the first contract, p. 103.

Figure IX.2: Brain Sodium in the White Matter of the Cerebral Hemisphere for Control Cats and Cats Injured at Different Energy Levels



Means ±5.0. ** p<0.01: * P<0.05 of Control + P<0.05. 0.9J v 1.43.

Note: This figure indicates the amount of brain sodium in the white matter of the cerebral hemisphere for control cats and cats injured at different energy levels.

Source: LSU's final report on the first contract, p. 61.

Table IX.6: Research Data on Cerebral Blood Flow in "Uncomplicated" Cats

	Control	1 min.	30 min.	60 min.	90 min.
MABP MEAN	112.4	150.9[a]	116.1	112.1	116.2
(±SE)	(3.7)	(9.4)	(2.9)	(6.2)	(5.4)
CPP MEAN	106.9	110.7	73.8[a]	73.6[a]	76.0[a]
(±SE)	(3.6)	(8.4)	(6.2)	(8.7)	(8.1)
ICP MEAN	5.4	40.1[a]	41.5[a]	38.5[a]	40.1[a]
(±SE)	(0.7)	(6.8)	(5.9)	(4.5)	(5.0)
WHOLE BRAIN CBF MEAN	33.6	37.5	32.1	28.4	30.1
(±SE)	(1.9)	(2.7)	(2.4)	(2.2)	(3.2)
WHOLE BRAIN CVR MEAN	3.2	3.1	2.4	2.5	2.8
(±SE)	(0.1)	(0.3)	(0.2)	(0.3)	(0.3)

Note: This table indicates measurements of whole brain blood flow and cerebral vascular resistance in "uncomplicated" cats wounded at 0.9, 1.4, and 2.4 J. [n=14]. These "uncomplicated" cats demonstrated significant regional CBF changes, however, both at 1 and 30 minutes after wounding. Increased 1-minute blood flows occurred in the right and left upper frontal poles, the left parietal area, the left upper occipital pole, and the right tectum.

[a]-$p<0.05$ compared to control period (-10 min.).

Source: LSU's first annual report on the second contract, p. 12.

Table IX.7: Research Data on Cerebral Blood Flow in "Complicated" Cats

	Control	1 min.	30 min.	60 min.	90 min.
MABP MEAN	114.4	162.6	122.1	110.1	128.0
(± SE)	(3.8)	(13.8)	(6.0)	(10.4)	(22.8)
CPP MEAN	108.3	110.3	52.1[a]	36.7[a]	46.9[a]
(± SE)	(4.0)	(8.7)	(9.7)	(9.7)	(12.3)
ICP MEAN	6.1	52.3[a]	70.0[a]	73.4[a]	82.1[a]
(± SE)	(2.2)	(13.1)	(8.9)	(7.2)	(13.2)
WHOLE BRAIN CBF MEAN	36.7	32.0	22.3[a,b]	19.5[a,b]	16.4[a,b]
(± SE)	(4.4)	(4.6)	(3.6)	(4.0)	(4.6)
WHOLE BRAIN CVR MEAN	3.5	4.1	3.9	4.4	•
(± SE)	(0.6)	(0.9)	(1.5)	(2.0)	•

Note: This table indicates measurements of whole brain blood flow and cerebral vascular resistance in "complicated" cats wounded at 0.9, 1.4, and 2.4 J. [n=9].

[a]-$p<0.05$ compared to control period (-10 min.).

[b]-$p<0.05$ compared to corresponding contralateral area.

Source: LSU's first annual report on the second contract, p. 10.

	Number of cats
Atypically high catecholamines @ control	1
CBF/CBF autoregulation	
(Multiple aspects of CBF after wounding)	
McKowen	45
Torbati	111
Total	**156**
Total used	**156**
With usable data	111
Remainder:	45
Physiological instability prewounding	12
Death following brain wound	15
Massive brain bleeding postwounding	12
Technical	6
Prostaglandins	
Total used	**57**
With usable data	36
Remainder:	21
Unsuccessful initial experiments	9
Bloody CSF postwounding	7
Overnight death	5
Evan's Blue Dye experiment BBB	
Dye injected prewounding	
Total used	**48**
With usable data	27
Remainder:	21
Died postwounding	20
Failed experiment	1
Dye injected postwounding	
Total used	**45**
With usable data	19
Remainder:	26
Died postwounding	22
Died after dye given	2
Uncertain	1
Failed experiment	1
Physiology	
Total used	**36**
With usable data	20

(continued)

Comments From the Department of Defense

DIRECTOR OF DEFENSE RESEARCH AND ENGINEERING

WASHINGTON, DC 20301-3010

10 SEP 1990

Mr. Lawrence H. Thompson
Assistant Comptroller General
Human Resources Division
U.S. General Accounting Office
Washington, DC 20548

Dear Mr. Thompson:

This is the Department of Defense (DoD) response to the General Accounting Office (GAO) Draft Report, "ARMY BIOMEDICAL RESEARCH: Concerns About Performance of Brain Wound Research," Dated July 10, 1990 (GAO Code 118263), OSD Code 8412. The Department concurs with some of the GAO findings and with the recommendations, but only partially concurs or nonconcurs with other findings.

As discussed in the enclosure, the research being conducted by the Louisiana State University under an Army contract was appropriately conceived and directed toward resolution of a significant military medical problem. The expert panel convened by the GAO to review the project agreed with that opinion, although they expressed some concerns about certain details involving the current research project. The panel nonetheless concluded that the work was of such value that it should be continued. The areas of concern identified by the panel were not deemed sufficient to terminate the work and each is discussed in the enclosure.

With regard to the recommendations, during September 1990, the Office of the Director, Defense Research and Engineering, will be reviewing the reports of Louisiana State University and the Army studies and other pertinent records concerning the project. At the end of that process, if it is determined that there are still substantial additional benefits to be gained, the Director, Defense Research and Engineering, will recommend to the Secretary that the project be continued. If that is the case and if the Congress permits the project to continue, the Director, Defense Research and Engineering will direct the Army to take appropriate measures to ensure that all scientific and administrative requirements of the contract are fully met.

GAO DRAFT REPORT - DATED JULY 10, 1990
(GAO CODE 118263) OSD CASE 8412

"ARMY BIOMEDICAL RESEARCH: CONCERNS ABOUT PERFORMANCE OF BRAIN WOUND RESEARCH"

DEPARTMENT OF DEFENSE COMMENTS

* * * * *

FINDINGS

- <u>FINDING A</u>: <u>Basis For Army Brain Wound Research</u>. The GAO reported that the Army Medical Research and Development Command conducts a medical research and development program designed to support the soldier in the field and meet other Army health needs. The GAO explained that the research focuses on (1) combat casualty care, (2) military disease hazards, (3) combat weapon systems hazards, and (4) chemical weapons defense.

The GAO reported that, in conjunction with the program, the Army entered into two successive contracts with the Louisiana State University School of Medicine for brain wound research. According to the GAO, the purpose of the Louisiana project is to enhance the understanding of brain wounds to enable combat physicians to effect better treatment with drugs. The GAO noted that the Louisiana State University research focuses on wounds caused by low energy missile and shell fragments and uses a trauma model consisting of an anesthetized cat shot in the brain with a specially designed gun. According to the GAO, the proposals indicate that cats were selected (1) because their brains have a ratio of grey to white matter comparable to that of human brains, (2) because cat brains are small in size, would not require large amounts of expensive radioisotope doses, and (3) because cats are readily available and relatively inexpensive. The GAO reported that the period of performance for the first contract was July 1, 1983 to December 31, 1985, at a total cost of $342,450. The GAO found that the follow-on contract was awarded on April 15, 1986, with a period of performance scheduled to run through September 29, 1991. According to the GAO, the cost for the second contract has increased from about $1.682 million to about $1.768 million. The GAO noted that, as of September 30, 1989, a total of about $1.201 million had been paid to Louisiana State University. (pp. 1-3, pp. 16-25/GAO Draft Report)

Now on pp. 1-2 and 12-17.

<u>DOD RESPONSE</u>: Concur. The Army did enter into two successive contracts with Louisiana State University to conduct research aimed at developing sound physiologic and pharmacologic methods to ameliorate the brain damage resulting from wounding. The scope of this study is

ENCLOSURE

DOD RESPONSE: Concur. Louisiana State University is accredited by the American Association for Accreditation of Laboratory Animal Care, which is an independent organization that promotes high standards of animal care. Given the unique nature of the work, it is to be expected that the research would have unanswered questions or areas which receive constructive criticism and suggest future research directions by a group of scientists reviewing it.

- **FINDING C**: **Questions About Control Of General Anesthesia**. The GAO reported that the panel identified concerns about the Louisiana State University research in some areas (also see Finding B). The GAO reported that, because the areas in which most of the panelists expressed concerns could affect research results, the GAO further reviewed those specific areas. To do so, the GAO noted that it consulted with five veterinary anesthesiologists. According to the GAO, one area where the majority of the panel members indicated concern was the management of anesthesia.

The GAO explained that, to compare wounded animals with other wounded and unwounded animals, the animals should be maintained at the same depth of anesthesia. The GAO further explained that some of the measurements critical to the outcome of the Louisiana State University study, such as cerebral blood flow and cerebral metabolism, are influenced by general anesthesia--with changes in blood flow and metabolism directly related to the anesthetic dose. The GAO reported that the veterinary anesthesiologists, therefore, believed that, unless the dose is precisely controlled, it is impossible to determine whether the pathophysiological changes are due solely to the injury or to a combination of the injury and anesthesia.

The GAO reported the anesthesiologists were in agreement that, with the particular anesthetic used and the method of administration in the Louisiana research (phenobarbital injected into the cats' abdominal cavities), the research was difficult to control. According to the GAO, the anesthesiologists saw no evidence in the documentation they reviewed that the dose of anesthesia was precisely regulated. In addition, the GAO observed that, because of the way the anesthetic was administered, the depth of anesthesia and the duration could vary during and between experiments. The GAO found that, for the most part, anesthesia records were not kept on individual animals used in the experiments, and, in those cases when records were kept, the doses actually given varied significantly and did not agree with the protocols. In this regard, the GAO found that the anesthesia doses and times they were administered were recorded for only about 20 to 25 percent of the animals used in the research. The GAO reported that, based on its review of the anesthesia records, the veterinary anesthesiologists doubted there was documented experiments. Thereafter, anesthesia induction and maintenace became routine and anesthetic records were maintained on all animals, except on those in which dose and animal weight records were irrelevant to the outcome of the experiment.

- **FINDING D:** **Questions About The Effect And Adequacy Of Postoperative Care**. The GAO observed that postoperative care also affects research results. The GAO explained that careful monitoring of postoperative care for animals allowed to awaken from anesthesia is important to obtain data relevant to research objectives and to help ensure appropriate recovery. The GAO reported the veterinary anesthesiologists emphasized that all aspects of post-operative care should be documented in detail to confirm that uniform treatment was provided to all animals. According to the GAO, however, the Louisiana State University research team stated they do not consider postoperative care, which occurs after the experimental period, as relevant to their research design or analysis.

The GAO found that, in general, project records were not maintained regarding the postoperative care given to animals recovering from the experimental period. In addition, the GAO reported that the anesthesiologists identified several factors that suggest deficiencies in the postoperative care, such as the lack of analgesics (pain relievers). The GAO pointed out that postoperative care is important in order to interpret physiological and behavioral changes that may be caused by experimental treatment--such as injury or by anesthesia or pain. According to the GAO, however, the anesthesiologists indicated they could not determine the adequacy of post-operative care from the information provided to them. The GAO concluded that questions about the management of post-operative care in the Louisiana State University project is another concern that raises doubts about the validity of some of the research results. (pp. 3-4, p. 7, p. 35, pp. 41-45, pp.70-71/GAO Draft Report)

Now on pp. 3-5, 21, 25-28, and 41.

DOD RESPONSE: Nonconcur. The DoD disagrees that post-operative care is relevant to the research design and analysis, since it occurs after the experimental period. All cats used in the Louisiana State University study were terminal; either they died as a result of the study or were euthanized for histopathological examination. The GAO also states that the documentation of postoperative care was not thorough. While the adequacy of postoperative documentation is a matter of interpretation, the attending veterinarian at Louisiana State University did maintain postoperative records indicating such treatments as the administration of parenteral fluids, antibiotics, and nutritional support. Those were the only records necessary for the purposes of determining the extent of neurological

DOD RESPONSE: Nonconcur. The other questions raised by the anesthesiologists do not affect the validity of the research results. The blood gases values that the GAO report refers to as being normal are for unanesthetized cats, which are being compared with values from anesthetized cats prior to wounding. Any general anesthetic would be expected to have varying degrees of respiratory depression. Thus, it would be expected that the blood gas values for the anesthetized cats would be out of the range determined for unanesthetized cats.

The Army awarded the first contract to Louisiana State University on the assumption that a valid model did not exist for studying fragment injuries and testing various treatment regimens. One of the many significant accomplishments from the initial contract was the development of the first such valid model. As with the development of any new model system in biomedical research, there are often significant numbers of initial failures. The high death rate reported by the GAO can be attributed to attempts to develop this new cat model and to the high risk of brain wounding, but should not be considered as reflecting negatively on the experimental design. Contrary to the comments of two of the veterinary anesthesiologists, the Louisiana State University cat model did yield graded responses. For example, the model demonstrated a direct correlation between the speed of the projectile with the percentage of cats dying from apnea and with an increase in intracranial pressure.

The GAO scientific panel felt that reporting of data was not an issue. Most, if not all, of the unreported data can be attributed to animals lost to technical problems with developing the new model. Accepted scientific procedures dictate that, if the animal dies before the experiment can be completed, then there are no data for that animal. In these instances, therefore, the data were not reported. Louisiana State University provided all animal information and data to the GAO investigators and there was no attempt on its part to conceal or selectively use any data.

- **FINDING F: Contract Compliance With Public Law 100-202.** The GAO reported that a portion of the funds for the current Louisiana State University contract with the Army were provided by Public Law 100-202, the DoD Appropriation Act for Fiscal Year 1988. The GAO explained that Section 8056 of that law states that " ...none of the funds appropriated by this Act shall be used to purchase dogs or cats or otherwise fund the use of dogs or cats for the purpose of training Department of Defense students or other personnel in surgical or other medical treatment of wounds produced by any type of weapon." The GAO concluded that, because the Louisiana contracts are research efforts and not training, use of cats in the project does not violate the GAO found that the contractor made scope and methodology changes to the research, without obtaining Army approval. The GAO noted that the panel of medical experts it convened commented that many of the changes improved the research effort. The GAO pointed out, however, that some of the methodological changes were in areas that experts had raised questions about--and many were made without getting prior written Army approval. The GAO concluded that the contract performance has been poorly monitored by the Army. The GAO further concluded that the poor monitoring is an indication that the Army management of the research projects has been inadequate. (p. 4, pp. 8-9, p. 56, pp. 58-67, pp. 71-72/GAO Draft Report)

Now on pp. 3-4, 6, 34-39, and 41-42.

DOD RESPONSE: Partially concur. The Army appoints individuals to function as contracting officer representatives for several contracts simultaneously. The current contracting officer representative for the brain wounding research project has been involved with it since 1986. While it is correct that other individuals were assigned technical monitoring responsibility prior to that time, the personnel changes that transpired were directly a result of unavoidable circumstances, including reassignment from the Command and retirement from Government service. During the periods between the departure of a technical representative and the appointment of a successor, the acquisition management liaison officer for the proponent laboratory assumed the responsibility for assuring that all technical issues were properly addressed by the appropriate source within the laboratory. When the second contract was awarded the laboratory commander decided that the acquisition management liaison officer would be formally appointed as the contracting officer's representative for all contracts, with actual technical monitoring being performed by the appropriate member of the scientific staff. Although the acquisition management liaison officer was, in fact, appointed to this capacity, the current contracting officer's representative performed the actual technical monitoring. The practice of appointing the acquisition management liaison officer as the contracting officer's representative for all contracts was discontinued in 1987, with the actual technical monitor now being appointed to that capacity.

The Army contract system encourages the contracting officer's representative to conduct annual site visits to each of his/her appointed contracts. In addition, the contracting officer's representative has telephonic discussions with the principal investigators concerning data or problems arising during the duration of the contract. Most, if not all, problems that arise can be handled via the telephone, which tends to minimize the importance of site visits. While site visits did not occur under the first contract, annual site visits were conducted

As an example, the GAO observed that changes to the anesthetic protocols would appear to at least have warranted an inquiry from the contracting officer representative about the reason for the change and how the change might affect research results. According to the GAO, the earliest indication that Louisiana State University had changed the anesthetic came in a report after work on the first contract was completed. The GAO further observed that an inquiry would, nonetheless, still have been relevant to determine the impact of the change on the second contract. In addition, the GAO pointed out that the Louisiana State University report also indicated the trauma model had limitations for drug testing. The GAO observed that, since drug testing was also an objective of the second contract, notice of the model's limitations was a reasonable basis for inquiry and assistance from the Army. The GAO concluded that technical assistance has not been provided by the Army when it might have been appropriate. The GAO further concluded that the lack of technical assistance is another indication that the Army management of the research projects has been inadequate. (p. 4, pp. 7-8, p. 56, pp. 68-69, pp. 71-72/GAO Draft Report)

Now on pp. 3-6, 34, and 40-41.

DOD RESPONSE: Partially concur. Unfortunately, due to lack of documentation, the level of technical assistance actually provided cannot be verified.

As previously stated, the principal investigator's decision to change the anesthetic agent at the start of the second contract was not a change in the contract's methodologies. The principal investigator had consulted experts in the field of anesthesia and head trauma as well as conducted a lengthy literature search before the decision was made to change anesthetic agents. Thus, the decision to change anesthetic agents was made properly and thoughtfully. We do agree, however, that the changes should have been discussed withthe Army before implementation. The research investigators have been advised to follow pre-consultation /pre-approval procedures before making any changes in the future.

Contrary to the GAO finding that the Louisiana State University reported that the trauma model had limitations for drug testing, the annual report dated April 27, 1989, stated "we have perfected a model to test drugs to try to improve neurological recovery after brain wounding." The principal investigator has not yet tested the six drugs specifically named in the original protocol because, in the six years since the protocol was written, several of these drugs are no longer applicable to the study. Instead, the principal investigator has chosen to test newly developed drugs that show a great deal more promise for treating

Now on pp. 6 and 42.

- **RECOMMENDATION 3:** The GAO recommended that if, after he reviews the project (see Recommendation 2), the Secretary of Defense finds it desirable to continue the project, the Secretary should ensure that the concerns identified by the GAO have been resolved. (p. 9, p. 72/GAO Draft Report)

  DOD RESPONSE: Concur. If the Secretary determines that the project should be continued and the Congress permits it, the Army will be directed to take all appropriate measures to ensure that the concerns identified by the GAO have been adequately resolved. This will include both administrative (such as fully documenting telephone conversations and pre-consultation/pre-approval on any contract change) and scientific concerns. The problem noted by the GAO concerning late reports has already been addressed by the Army and the implemented procedure is working effectively. The Army recognized that changing contracting officer representatives could be a detriment to any biomedical contract. The Office of the Director, Defense Research and Engineering, has encouraged the Army Medical Research and Development Command to keep reassignment of contracting officer representatives to a minimum. The Office of the Director, Defense Research and Engineering, will monitor future progress of the project with periodic reviews to ensure compliance.

Comments From Louisiana State University

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LSUMC

Office of the Dean

September 7, 1990

Ms. Linda G. Morra
Director
Intergovernmental and
Management Issues
U.S. General Accounting Office
Washington, D.C. 20548

Dear Ms. Morra:

I enclose our response to the Draft Report response to your document about Dr. Michael Carey's research. We are pleased that you will include our response in your final report. We also appreciate the 30 day extension to September 10.

Sincerely,

Robert S. Daniels, M.D.
Dean

RSD:cvr
Enclosure

School of Allied Health Professions
School of Dentistry
School of Graduate Studies
School of Medicine in New Orleans
School of Medicine in Shreveport
School of Nursing

LSUMC RESPONSE TO DRAFT GAO REPORT RECEIVED 7/10/90
"CONCERNS ABOUT PERFORMANCE OF BRAIN WOUND RESEARCH"

Attachments: **Exhibits**
References

1. SUMMARY

This document, including exhibits 1-7 and references, comprises Louisiana State University Medical Center's response to the GAO draft report on Dr. Michael E. Carey's brain wound research project. Louisiana State University Medical Center (LSUMC) is gratified that the GAO's expert Scientific Review Panel not only vindicated Dr. Carey's research project on brain wounds--supporting the validity, uniqueness and importance of this work--but also strongly recommended its continuation and continued funding. This knowledgeable panel wrote pages of positive remarks including multiple comments regarding: the importance of the work, the reproducibility of the model, the validity of the results, the immediately transferable information to brain-wounded humans, the impressive facilities, the necessity of using live animals, the absence of other laboratories performing such studies, the appropriateness of care of the experimental animals, the great potential for treatment improvements for humans, the outstanding qualifications of the investigational team, and the significant contribution this research has made to the body of information available in this area.

Significantly, this project has undergone three separate and extensive reviews--by the American Association of Neurological Surgeons (AANS), the LSU Faculty Review Committee, and by the GAO Scientific Review Panel--with essentially the same conclusions: that the research is unique, valuable, has been well and humanely done, and should be continued because it may save human lives.

2. RESEARCH INVESTIGATOR

The principal investigator, Michael E. Carey, M.D., Professor of Neurosurgery at Louisiana State University Medical Center in New Orleans, is a practicing neurosurgeon. He was a combat neurosurgeon in Vietnam where his neurosurgical unit operated on more than 300 people with brain wounds. During his 22 years of Army service, Dr. Carey has been awarded the Bronze Star, Purple Heart, the Army Commendation and the Humanitarian Medal. The South Vietnam Government presented Dr. Carey the Technical Service Medal First Class.

3. HISTORY OF THE PROJECT AT LSUMC

Following his experience as a MASH neurosurgeon, Dr. Carey was appalled to learn from the neurosurgical literature that essentially the same percentage of young men died from brain wounds in Vietnam as in World War II (WWII mortality 11%-14%; VN mortality 10%-12%) (1). Contrary to the progress made in other areas of medicine during this fifty-year span, the treatment of brain wounds had virtually stood still. Dr. Carey wanted to change that. While this was his original motivation, it has become increasingly apparent that Dr. Carey's work is needed even more urgently by our civilian population with violent crime on the rise. It can be inferred from statistics published in 1983 that 16,000 Americans die on city streets each year from gunshot wounds to the brain. Furthermore, more than 70,000 serious brain injuries occur yearly in the United States; and the cost of caring for these individuals is $25 billion per year. Since so few laboratory papers on brain wounds have been published, almost nothing is known about it in modern, scientific terms. Having published more than a dozen clinical papers on brain wounds and wanting to improve the plight of those with brain injury, Dr. Carey knew that the problem should be taken to the laboratory where it could be understood and new treatments devised. One promising area of treating brain wounds has yet to be explored--experimental drugs which, when tested, might prove effective in limiting the damage following brain injury.

For technical and scientific reasons, Dr. Carey elected to use the cat for his laboratory model. The cat has been used in neuroscience studies for more than 100 years with much information on brain function already worked out--information which has been and is routinely applied to humans.

Under Army auspices, Dr. Carey established the only laboratory in the world studying the effects of brain wounds in order to help people survive them. From 1984 to 1990, Dr. Carey's laboratory published 5 papers and 17 abstracts.

At the behest of Representative Robert Livingston, the GAO began its investigation of Dr. Carey's project in December of 1988. It convened an expert Scientific Review Panel chaired by John Jane, M.D., Professor of Neurosurgery and Chairman of the Department of Neurosurgery at the University of Virginia at Charlottesville, to review Dr. Carey's research project. The GAO Scientific Review Panel included seven distinguished scientific authorities with unquestionable expertise in neuroscience, trauma, physiology, and animal care. This panel concluded that Dr. Carey's research was unique and valuable and that its funding should continue.

LSUMC has been told that after the expert Scientific Review Panel reached its conclusions, the GAO then contacted five veterinary anesthesiologists to evaluate the project. These particular veterinary anesthesiologists are not recognized experts in brain trauma thus, have questionable credentials to evaluate a brain research project. To LSUMC's best knowledge, the veterinary anesthesiologists did not interact with the expert Scientific Review Panel. They also had no interaction with Dr. Carey. The interactive process is a necessary and customary part of a valid scientific review. The veterinary anesthesiologists apparently did not submit a written report. All that appears in the GAO draft is what the GAO wrote and repeated attempts to secure a copy of any report authored by the veterinarians failed to produce one.

The LSU Faculty Committee, comprised of top LSU scientists with national and international reputations, was convened by Dr. Allen Copping, President of the LSU System, to evaluate Dr.

Carey's research in October, 1989. The LSU committee concluded that Dr. Carey's research addressed critical scientific problems, was well-conceived, and made maximum effort to protect the welfare of animals. The LSU Committee strongly recommended its continuation (Exhibit 1).

The American Association of Neurological Surgeons, the principal spokesgroup for the U.S. neurosurgery community, also evaluated Dr. Carey's research. An AANS committee of distinguished neurosurgeons had access to the same material as did the GAO and spent a full day visiting Dr. Carey's laboratory where questions were asked regarding the project. The AANS concluded that Dr. Carey's research is being carried out by an investigative team that has the background, physical facilities and equipment to conduct such research and that Dr. Carey's team is the only one currently studying brain missile injury in the U.S.; that the work that has been done and is planned does not duplicate the comparatively little research previously conducted and reported in this area; that the anesthetic technique is humane and does not invalidate the experimental modeling; that the experimental model is appropriate; that a computer model cannot reproduce the widespread response to injury necessary to understand and treat this type of wound; that Dr. Carey's studies have been well-planned and well-executed; and that the model is developed to the point where treatment trials can be initiated to search for ways of improving the outcome from missile injuries to the brain. The AANS recommended that Dr. Carey's research be continued (Exhibit 2).

In summary, the GAO Scientific Review Panel, the LSU Faculty Committee, and the American Association of Neurological Surgeons, having all examined Dr. Carey's research project in depth, have all reached the same conclusions: that Dr. Carey's work is needed; that the research has been done well; that the research has been done humanely; that the research findings can be applied to help save human lives; and that the research should be continued.

4. LSUMC CONCERNS REGARDING THE CONDUCT OF THE GAO REVIEW

In LSUMC's opinion, the GAO review of the project has been biased against the research. Its draft report is weighted heavily to negative comments, while in reality, the creditable (Scientific Review Panel) review was positive. Some of LSUMC's major concerns include:

A. GAO Reported Inaccurate Information & Inadequately Transferred Information

The GAO constructed a review process that did not allow an exchange of information between qualified scientists. The Scientific Review Panel was not permitted to visit Dr. Carey's laboratory to see his data firsthand or to question Dr. Carey. Had they been allowed access to Dr. Carey, they would have caught some of the mistakes the GAO made in reading and trying to interpret technical and complex scientific data. The Scientific Review Panel worked solely from information provided by GAO staffers whose expertise is not in science. They went through Dr. Carey's laboratory selecting information for the scientists. The GAO's interpretations of Dr. Carey's data and information were sent, along with Dr. Carey's Army reports, to the Scientific Review Panel. The GAO also wrote a list of questions to guide the review.

It should be understood that some of the information the GAO sought included false claims made by animal activists about Dr. Carey's work. The GAO admittedly met with representatives of the Physicians' Committee for Responsible Medicine (a small but vocal animal activist group that has no connection to the professional medical community and that is not only opposed to any use of animals in biomedical research but is also actively working to stop research projects that use animals).

The Scientific Review Panel had no way of knowing that some of the information provided by the GAO was in error. For example, the GAO reported that Dr. Carey had performed 33 types of experiments when his data clearly indicated that there were only nine areas of research interest. Similarly, the Scientific Review Panel (GAO draft Appendix IV) spent time discussing "multiple anesthetics" purportedly used in the experiments when, in reality, only one anesthetic, pentobarbital, was used for all completed research projects bearing on brain physiology. Dr. Carey did initially test several anesthetic agents before determining that pentobarbital was the best one to use, but these early tests using a small number of animals were misrepresented by the GAO and given the same weight as Dr. Carey's main experimental studies. Also, the GAO Scientific Review Panel needed more information about the techniques for studies proposed by Dr. Carey in his Contract Proposal. Had he been at their meeting in June of 1989, Dr. Carey could have explained that he worked under a time restriction of less than 60 days and could only take 30 pages to prepare and outline protocol for a 5-year research project. Naturally, specific projects could only be indicated in outline form. Dr. Carey also could have provided the panel a manuscript and abstracts for inclusion in the panel's considerations which would have eliminated many questions that now appear in the body of the GAO Scientific Review Panel report, (e.g., concerns about anesthesia, details of some of Dr. Carey's experimental techniques, and particularly the question of productivity). The GAO did ultimately provide the Scientific Review Panel with that manuscript and those abstracts, but not until after the panel had met.

Louisiana State University Medical Center would like to emphasize, however, that in spite of the obstacles placed before it, the GAO Scientific Review Panel still managed to reach a conclusion: that Dr. Carey's research had merit, was needed, and should be continued.

B. Inappropriate Exit Interview by the GAO

The "exit interview" conducted by the GAO in September, 1989 at LSU with Dr. Carey did not provide a mechanism for appropriate response to GAO concerns. Contrary to accepted scientific practice, and especially in light of such voluminous technical data, written questions were not provided in advance of the "exit interview"; Dr. Carey was expected to respond extemporaneously. For example, the question of his use of pentobarbital anesthesia in the experiments could have been completely clarified by inclusion of more than 100 publications drawn from the scientific literature which affirm Dr. Carey's choice of pentobarbital.

C. Inappropriate Anesthesiology Review

Inclusion of the opinions of the individual veterinary anesthesiologists is a major concern. Richard Traystman, M.D., Chief of Anesthesia Research at Johns Hopkins Medical School and a world authority on the cerebral effects of anesthesia, was on the GAO Scientific Review Panel; since the expert panel did not find that pentobarbital was an inappropriate anesthetic, it is unclear why the GAO sought the evaluation by the veterinary anesthesiologists, particularly these veterinary anesthesiologists. Had the GAO acted responsibly, it would have turned to research anesthesiologists with worldwide reputations in brain physiology. A list of more than 120 recent publications authored by the five selected veterinarians fails to show any papers on brain physiology or brain injury. While these veterinarians are distinguished in their respective fields, they have no generally recognized expertise in brain physiology, brain injury, brain blood flow, or brain metabolism. It is, therefore, inappropriate to have these nonexperts in brain research comment upon the intricacies of this research.

The veterinary anesthesiologists, like the expert Scientific Review Panel, were also apparently given incomplete and misinterpreted data. For instance, the veterinary anesthesiologists were evidently informed that Dr. Carey anesthetized the animals by intraperitoneal pentobarbital when, in fact, anesthesia was generally induced with intraperitoneal pentobarbital but maintained by intravenous pentobarbital--a significant difference. This anesthetic technique is commonly used (10, 12, 13).

It is of great concern that, to the best of LSUMC's knowledge, these consultants were interviewed individually rather than by a consensus development method more consistent with accepted scientific review practices and that they evidently did not prepare a written report.

D. Inappropriate GAO Emphasis in the Draft Report

Considering the absence of appropriate qualifications of the veterinary anesthesiologists to review a brain research project and their questionable method of review, it is unconscionable that the GAO draft attached more significance to the individual comments of the veterinary anesthesiologists than to the carefully considered evaluation of the GAO Scientific Review Panel which was appointed by the GAO itself.

The comments of the GAO Scientific Review Panel were written and widely circulated among its members. Opinions expressed in writing were collated into a consensus paper by Dr. Jane and represent an objective, informed evaluation of the brain injury project.

Unlike its own expert scientific panel's report, the GAO draft report is written with bias against Dr. Carey's research and fails to note positive comments about Dr. Carey's research. Although the Scientific Review Panel contained numerous positive comments, they are not mentioned at all in the Executive Summary nor in the body of the draft written by the GAO. The fact that the GAO Scientific Review Panel felt that "the project has merit and should receive continued funding" is downplayed in the Executive Summary. Under the heading, "Principal Findings," the only reference to one of the Scientific Review Panel's most important findings--that the project has merit--is relegated to a headline. Conspicuously absent from the "Principal Findings" is the Scientific Review Panel's main finding that Dr. Carey's research should be continued.

Although its own Scientific Review Panel's report was extremely favorable, the GAO chose to largely ignore it. The GAO's Executive Summary and Chapters 1-4 instead dwell on the individual concerns of the veterinary anesthesiologists contacted by the GAO after the its Scientific Review Panel had met. The GAO replaced the positive, substantive comments concerning Dr. Carey's research made by the GAO Scientific Review Panel (which has immense expertise in brain research) with critical comments made by the veterinarians (who have no generally recognized expertise in brain physiology or brain trauma).

5. RESPONSE TO CONCERNS CONTAINED IN THE GAO DRAFT

LSUMC is disturbed that the GAO draft focused on negative, peripheral, and erroneous concerns. In the interest of truth, it is important to address these issues. Since this section contains detailed scientific information, it will be necessarily lengthy and complex. This length should not be construed as being indicative of the importance assigned to these concerns by LSUMC.

A. Anesthetic Issues

The task of choosing the anesthesia to be used in these experiments was not undertaken lightly by Dr. Carey. After careful study of various anesthetic possibilities in the scientific literature, Dr. Carey chose pentobarbital for studies concerning brain edema and cerebral blood flow. Pentobarbital has been widely used in such studies and in studies on brain trauma. While pentobarbital may have some "cerebral protective effects," it is probably not a free radical scavenger (2). Even if barbiturate anesthesia does offer a modicum of brain protection, any such effect would be accounted for, including drug-testing experiments, by comparing pentobarbital-anesthetized, brain-wounded, control animals with pentobarbital-anesthetized, brain-wounded, drug-treated animals. In testing drugs to determine their ameliorating effects in brain wounds, Dr. Carey initially sought a shorter-acting anesthetic (isoflurane), but it produced an unacceptable post-wounding mortality. He, therefore, decided to use pentobarbital anesthesia even for drug testing, accepting the prolonged anesthetic effect of pentobarbital on the cat. Using one anesthetic for all experiments (acute-physiological and chronic-recovery) has the decided advantage that all physiologic, behavioral, and biochemical results can be understood from the viewpoint of one anesthetic. Dr. Carey was well aware of the limitations of pentobarbital anesthesia and stated in discussing apnea observed after missile wounding: "Whether the observed apnea would be significantly modified by use of another anesthetic agent......is unknown" (3). References (4-27) document studies of renowned investigators who also used pentobarbital as anesthetic in studying brain edema or various aspects of brain blood flow in cats. Dr. Carey sought additional opinions from world-recognized authorities in brain research who supported the choice of pentobarbital for these experiments (Exhibits 3, 4, 5). Pentobarbital is an appropriate and commonly used anesthetic choice for laboratory research on brain trauma, brain edema, the blood brain barrier, and cerebral blood flow.

The GAO draft states, "The anesthetic and its method of administration in the research--pentobarbital administered intraperitoneally (IP. . .) made controlling the depth of anesthesia difficult." That statement is not exactly accurate. While anesthesia was generally introduced intraperitoneally, intravenous supplementation of anesthetic was done as required so that each animal's corneal response was abolished and the animal would not respond to paw pressure. Four documents (28-31) to which the GAO had access clearly state that IP pentobarbital was used only for induction, followed by intravenous (IV) anesthetic for maintenance as is often done in brain research (10, 12, 13). In some animals, anesthetic was induced as well as maintained by IV pentobarbital.

The GAO draft report also states "it is extremely important that general anesthesia be administered in a careful and controlled manner so that the reactions of study and control animals can be compared. In this way any changes that occur will be the result of the trauma rather than the anesthetic." Dr. Carey did just that: control, unwounded cats were anesthetized with pentobarbital in exactly the same fashion and subsequently treated exactly as the experimental, wounded cats except that no brain wound was inflicted. In this way, he could measure physiological and behavioral variables under the influence of pentobarbital anesthesia alone and compare these variables to those measured in the pentobarbital-anesthetized, brain-wounded cats.

Inhalant anesthetics were not used for these experiments because the commonly used volatile anesthetics uncouple the relationship between blood flow and metabolism, and all interfere with brain blood flow regulation (Exhibit 3).

Finally, if poorly controlled anesthetic techniques would be expected to adversely affect "physiological parameters critical to the outcome of the LSU research," one would expect the achieved LSU cerebral blood flow results to be markedly different from results given in the literature. This is not the case indicating that anesthesia was well-controlled.

Table 1
Total Cerebral Blood Flow Determined in
Pentobarbital-Anesthetized
Cats by Various Investigators

Individual Investigator	Total Cerebral Blood Flow (ml/100g/min)
McKowen (29) LSU	33-36
Torbati (30) LSU	36-39
Dewitt (11) MCV	30-36
Davis (13) Mayo Clinic	34-50
Zierski (19) Giessen	38
Risberg (22) Lund	40

Table 1 shows that the LSU data are well within the range of four other major brain research laboratories.

B. Postoperative Analgesia

Only about 13% of all cats used in the experiments were allowed to survive the acute experiment. Thus, the consideration of postoperative-wounding pain in Dr. Carey's research applies to a relatively small number of animals. The postoperative-wounding protocol was carefully considered by the LSU Institutional Animal Care and Use Committee and by contract reviewers. Both of these authorities deemed that the decision not to use postoperative-wounding analgesics was correct. Topical analgesics, which do not interfere with brain function, were used. Not only did the common clinical, human experience suggest that pain after brain injury would be negligible, but analgesics given in the recovery period might seriously interfere with judging whether an experimental drug might improve brain function. All cats allowed to recover were watched intently by Dr. Carey, his research team, and members of the Division of Animal Care. The members of the Division of Animal Care are acutely attuned to discomfort in animals and are bound by professional responsibility to note and report any observed animal suffering. None was reported to Dr. Carey or Dr. Gonzalez, Head of the Division of Animal Care.

In attempting to ascertain if there was postoperative pain, the GAO compares cats with brain wounds to humans with pseudotumor cerebri (a condition where the brain swells). This is an incorrect analogy; cats with brain wounds should be compared to humans with brain wounds. Neurosurgeons recognize that in humans, pain is not a problem following brain trauma, including brain missile wounding. Failure to perceive pain is especially true if the brain-injured person is drowsy or in coma following brain injury. Under these conditions, drugs such as morphine or meperidine, which are commonly used to relieve pain, are contraindicated because they also decrease respirations and may kill the patient. Dr. Carey's experimental animals were drowsy for approximately two days. On the grounds of good human neurosurgical practice, analgesics are not given.

In a book about animal pain, Hughes and Lang (32) state, "The perception of pain is an extremely complex physiological phenomenon. Animals may have a higher pain threshold than humans undergoing similar procedures. Therefore, it is important to temper judgment of a painful experience with careful observations of animals and the individual response to the stimuli." Observations of Dr. Carey's experimental animals indicated that they did not have significant pain after surgery/brain wounding. He, therefore, had no reason to treat them for pain. Significantly, Hughes and Lang do not recommend that animals running about on the day of surgery following a hysterectomy (a more painful procedure than brain surgery) be treated with analgesics. They do not need them. There is no question that the decision to not provide postoperative analgesia was appropriate.

Several of the veterinary anesthesiologists evaluating Dr. Carey's research have not provided any postoperative pain for goats, dogs, or cats undergoing abdominal surgery or for awake cats with indwelling femoral artery cannulae (33-36). Since some of the very same veterinary anesthesiologists who criticized Dr. Carey's decision not to use analgesics do not find it necessary to give their postoperative cats analgesics, their criticism of Dr. Carey is inconsistent.

The GAO wrote an erroneous statement in its draft under the section on "Postoperative Pain." It states, "the [LSU] veterinarian [Dr. Longoria] told us [GAO investigators] that the animals from the brain wound project experienced pain. He also told us that he treated them for the pain with Butorphanol Tartrate, an analgesic drug." This statement misrepresents the facts. Dr. Longoria categorically denies that he told the GAO that Dr. Carey's cats experienced pain (Exhibit 6). In fact, he wrote a letter (Exhibit 7) about this issue stating that only one cat received Butorphanol--for an ear infection--long before any experiment. Dr. Carey gave that letter to the GAO in November of 1989.

C. Postoperative Care

After wounding, cats were suctioned through the endotracheal tube, given antibiotics, and kept covered with a thermostatically controlled heating blanket controlled by a rectal thermometer. Vital signs (blood pressure, heart and respiratory rates) and end tidal CO_2 were continually monitored; blood gases were intermittently measured. Pupillary size was also periodically determined. When the animal appeared to be stable, all cannulae (including the femoral artery line) were removed, and this artery was tied off. The groin wound was closed. Collateral arteries provide sufficient blood supply to the leg, so problems from ischemia did not occur. The cats were subsequently returned to the animal care facility, covered with a blanket, and the veterinarian was informed that injured cats had been returned. Injured cats were observed intently by a member of the investigative team. The following morning the cats were usually still unconscious. Pupillary size and respiratory rate were checked and the warming blanket maintained. On post-experiment day one, the veterinarians routinely administered lactated Ringer's solution IP, antibiotics IM (intramuscularly) and nutritive support. The cats were also checked regularly that day by a lab member. On day two post-experiment, the animals were checked exactly as for day one. The animals were usually groggy and ataxic. If the cats were not observed to be eating and drinking, the veterinarian was notified and lactated Ringer's, antibiotics, and nutritive support were administered. On day three post-experiment, the animals were checked for pupillary size and reaction, respiratory rate, and also to see if they were eating and drinking. Most were eating and drinking ad lib by this time, but if not, lactated Ringer's solution, antibiotics, and nutritive support were again administered by the veterinarian. By day four post-experiment, all cats were eating and drinking ad lib. No indication of "apparent" pain was present at any time. If any had been, the veterinarian or his staff would have notified Dr. Carey. This never occurred. The quality of animal care is unquestioned and is documented by the fact that the LSUMC animal care facilities and programs are accredited by the American Association of Animal Laboratories and Animal Care (AAALAC).

D. Blood Gas Issues

The veterinary anesthesiologists (but not the GAO Scientific Review Panel) questioned some of the arterial blood gas measurements reported in the 1985 yearly report (28). While cerebral blood flow (CBF) responds to relatively small changes in the arterial content of carbon dioxide ($PaCO_2$), CBF is only affected by extreme changes in the arterial content of oxygen (PaO_2)--not within a range of 60 mmHg to 150 mmHg. Thus, in studying CBF, $PaCO_2$ must be maintained within a physiologic range and be closely monitored; it is much less important to maintain PaO_2 to the "norm." So unimportant is the actual level of PaO_2 to CBF and cerebral metabolism studies that many research scientists take PaO_2 measurements only to insure that the experimental animal is not hypoxic (too little oxygen). In many research projects, actual PaO_2 levels are often either not reported at all (11, 13, 19) or stated only as being above a certain reasonable range (12). Dr. Carey did, however, record actual PaO_2 levels to assure that his cats were adequately oxygenated. The PaO_2 levels were well within acceptable range. Even though PaO_2 levels have little bearing on CBF, Dr. Carey's PaO_2 levels were questioned by the veterinary anesthesiologists; so their significance must be further clarified.

According to the scientific literature (4, 15, 17, 18, 24), various experiments on CBF or brain metabolism have reported mean PaO_2s ranging from 84 mmHg to 150 mmHg. Control PaO_2 values reported in Dr. Carey's experiments ranged from 60.8 mmHg to 127.5 mmHg with a mean of 97.7 mmHg. It can be calculated that Bose (4) observed PaO_2 values as low as 63 mmHg while Wei and Kontos (15) reported PaO_2s as high as 127 mmHg in pentobarbital-anesthetized, room air-breathing cats. Solter and Haskins (one of the consulting veterinary anesthesiologists), who specifically attempted to determine normal blood gas and pH values in awake cats, found a mean arterial PaO_2 of 85mmHg and observed PaO_2s as low as 73 mmHg. Dr. Carey's PaO_2 data are well within the range reported by many other investigators and also by one of the GAO's critical veterinary anesthesiologists. To say that the arterial PaO_2 in the air-breathing cat must lie between 95 and 100 mmHg does not conform to published PaO_2 levels. While the GAO report mistakenly states that 14 of 15 animals had an oxygen level outside the normal range, in reality, 14 of 15 PaO_2s in these cats were well within the reported range. It is quite reasonable to expect that some cats in Dr. Carey's experiments would have lower PaO_2 levels because these cats were not awake but pentobarbital-anesthetized. Anesthesia can depress respirations and may also cause pulmonary atelectasis (collapse of the lung) which would decrease the PaO_2. The reported PaO_2 levels may concern the veterinary anesthesiologists but not Dr. Traystman, Professor of Anesthesia Research at Johns Hopkins and a member of the GAO Scientific Review Panel, or Dr. John Michenfelder, Chairman of the Division of Anesthesia Research at Mayo Medical School, who looked at the same data. The technical point of satisfying the mathematical relationship between alveolar and arterial oxygen content is physiologically irrelevant because brain blood flow and metabolism are insensitive to PaO_2 over wide ranges.

$PaCO_2$, on the other hand, has a direct and important influence on CBF. It should be noted at this point, however, that when the scientists in Dr. Carey's laboratory were gathering the physiologic data presented in table 2.1 (GAO Report), they were not measuring cerebral blood flow or metabolism. They were measuring several physiologic variables including arterial blood gases and pH to determine the effects of brain wounds on respirations and other physiologic variables. To use data from one study to evaluate an entirely different study is inappropriate.

The GAO report implies that $PaCO_2$ values are out of the normal range (38-42 mmHg), and this may invalidate Dr. Carey's results. That this $PaCO_2$ range is "normal" is doubtful because awake cats may have a $PaCO_2$ as low as 30.5 mmHg (36). Since cats used in these particular experiments were pentobarbital-anesthetized and spontaneously breathing and many had depressed respirations, it is not surprising or significant that the $PaCO_2$ levels in some were elevated above "normal." The mean $PaCO_2$ for all 15 cats, however, was 39.7 mmHg--in the "normal" range. Whether the control $PaCO_2$ (or PaO_2) was slightly high or slightly low was immaterial as it was the post-wounding pattern of response that was important to evaluate. Dr. Carey's work clearly demonstrates that after brain wounding PaO_2 falls and $PaCO_2$ rises.

Because $PaCO_2$ is important relative to cerebral blood flow and because $PaCO_2$ may indicate adequacy of anesthetic and respiratory control, the $PaCO_2$ values used during the later CBF measurements and presented in the 1987 and 1988 reports are shown in Table 2. No question exists about the adequacy of this $PaCO_2$ data derived from CBF experiments.

Table 2
Arterial $PaCO_2$ in Pentobarbital-Anesthetized Cats in CBF Studies

Investigator	Institution	$PaCO_2$ (mmHg)
McKowen (29)	LSU	32.4
Torbati (30)	LSU	31.1-32.7
Dewitt (11)	MCV	27.4-31.2
Davis (13)	Mayo Clinic	39-41
Zierski (19)	Giessen	31.3

The LSU $PaCO_2$ measurements are within the observed range of three highly respected scientific investigators.

In summary, the $PaCO_2$ values in spontaneously breathing, pentobarbital-anesthetized cats presented in the first yearly report are within normal ranges used for brain function studies or are understandably slightly high owing to depressed respirations and CO_2 retention. The $PaCO_2$ levels in the CBF experiments are well within the ranges used by established investigators who also measure CBF in pentobarbital-anesthetized cat and indicate that the anesthesia was well-controlled and Dr. Carey's CBF experiments were well-done.

It is significant that the literally thousands of measurements Dr. Carey and his group reported on brain electrolytes and brain blood flow, his main research areas, were not questioned.

E. Data Reporting Methods

The GAO draft report states, "The reported results do not discuss data from experimental failures." The reasons for exclusion of animal data appear in Appendix XI of the GAO draft. Data from dead animals were not and should not be reported in results because the objective of Dr. Carey's research is to study and to understand nonfatal brain wounds. No useful purpose is served in studying fatal, nontreatable brain wounds. Exclusion of such data is necessary, customary, and ordinary for any reliable scientific analysis of performed experiments.

Appendix XI (GAO report) indicates the reasons animals were excluded from blood flow studies. For instance, to include cats with massive bleeding and shock would have clouded the effect missile wounding alone had on the brain. This would have provided data on the effect of missile wounding plus hemorrhagic hypotension (shock). Indeed, to have included such data would have been misleading.

F. Failure Rate Issues

Failure rate issues were never raised by the GAO Scientific Review Panel but, rather, by the veterinarians who have no denonstrable experience in either brain physiology or brain trauma research.

Dr. Carey's laboratory developed this entire model system to study brain wounds because no other facility like it exists in the world, and no other investigator has undertaken such an extended study of brain wounds. His laboratory developed and tested virtually all aspects of this model system before applying it to the physiological question under consideration--from developing the apparatus and selecting the wound trajectory to trying several types of anesthesia, working out biochemical assays, and running small pilot studies (e.g., for brain catecholamine assays).

A brain wound is a serious injury which up to now has resulted in a staggering percentage of deaths. Improved survival rates and a higher quality of life for survivors of brain wounds can only be achieved by thoroughly understanding what happens to the brain when it sustains a wound. Only then can the devastating or fatal effects of brain wounds be prevented or reduced. This is why Dr. Carey chose to study brain wounds: to understand and influence the process of brain wounding to save human lives.

Since there is so much more to be learned about brain wounds, and since a brain wound is often fatal, it is remarkable that Dr. Carey was able to develop a model that has proven so successful. On average, it has yielded useful data in two out of three experiments.

G. Graded Response of Trauma Model

Two of the veterinary anesthesiologists felt that the brain wound model which Dr. Carey developed lacks a graded response to missiles of differing energy. The GAO then states that "a graded response model, such as this one, should demonstrate progressive and statistically different responses for injuries inflicted at different levels of energy (missile impact)." In the two most fundamental aspects of brain injury, the apneic response and the sustained elevated intracranial pressure, the model clearly demonstrates a graded physiological response to missiles of increasing energy (3, 28, 30) as shown in Table 3.

Table 3
Effect of Missile Energy upon Fatal Apnea (Apnea>6 minutes)
And Sustained Increase in Intracranial Pressure

Missile Energy (Joules)	% With Fatal Apnea	Intracranial Pressure 30 min. post-wounding (mmHg)	
		Sarna, 1985	Soblosky, 1989
0.9	8.6	21	17
1.4	38.9	29	35
2.4	66.7	43	50

H. Late Reports

Dr. Carey accepts responsibility for any late reports. In fulfillment of his contract each year, he has turned in to the Army from 82-100 pages of data and interpretation of results. Collation and interpretation of the immense amount of data (e.g., approximately 3000 brain water and electrolyte measurements and 5000 regional blood flow determinations) require time, consideration and discussion among the research team.

6. CONCLUSIONS AND SIGNATURES

Louisiana State University Medical Center believes Dr. Carey's research on brain wounds should continue. One can hardly envision an investigator's work undergoing a more stringent or lengthy review process. In the course of three separate investigations--by the GAO's own expert Scientific Review Panel, the American Association of Neurological Surgeons, and the LSUMC Faculty Review Committee--Dr. Carey's work has been unanimously found to be greatly needed, to have merit, to have documented an extensive record of accomplishment, to have been conducted in an excellent research environment by an exceptionally qualified research team, to have been conducted humanely; and its continuation has been unanimously recommended. Dr. Carey's research has already produced knowledge that can be used to treat people with brain wounds and is on the threshold of discoveries that have the potential to save thousands of human lives. It would be most ironic if, in this the presidentially designated "Decade of the Brain," a research project uniquely qualified to cross a last frontier of medicine were to be stopped.

LSUMC knows of no other medical scientist who has been exposed to such a politically motivated and controlled process. The GAO review process is at variance with accepted standards of the peer review process for scientific research. The Secretary of Defense and others should weigh the effect on the country's scientific community if such an aberrant review is allowed. A dangerous precedent may be set which will affect not only this medical research project but many others to follow. Unfortunately, not only will medical progress be seriously jeopardized, but innocent American people will pay the price with their health and well-being and many with their lives.

Robert S. Daniels, M.D.
Dean
LSU School of Medicine

Perry G. Rigby, M.D.
Chancellor
LSU Medical Center

Michael E. Carey, M.D.
Professor of Neurosurgery and Principal Investigator
LSU School of Medicine

EXHIBIT 1

SCHOOL OF
MEDICINE IN NEW ORLEANS
Louisiana State University
Medical Center
1542 Tulane Avenue
New Orleans, LA 70112-2822
Telephone: (504) 568-4006

Office of the Dean

March 7, 1990

Executive Committee
Faculty Assembly
LSU School of Medicine

Dear Committee Members:

President Copping recently appointed a Committee of distinguished LSU faculty members to review the research project, "An Experimental Brain Missile Wound' Ascertaining Pathophysiology and Evaluating Treatments to Lower Mortality and Morbidity". I attach the report.

The project has been under investigation for more than a year at the request of Congressman Livingston to the General Accounting Office. Although the last site visit occurred in September and the last visit with GAO staff occurred in November, a report has not yet been issued. Also, in November, Congressman Livingston attached an amendment to an appropriations bill to terminate funding temporarily until 30 days after the GAO report appeared.

We believe that this situation is one to which the biomedical scientific community and the public must attend because of its potential public policy consequences. We urge your careful review, your support in urging the GAO to issue its report, and your attention to the possible consequences.

Sincerely,

Robert S. Daniels, M.D.
Dean

RSD:cvr
Enclosure

EXHIBIT I

REPORT OF THE FACULTY COMMITTEE TO REVIEW
DR. M. E. CAREY'S RESEARCH PROJECT

Dr. Allen Copping, President of the LSU System, directed that a committee be formed to conduct a review of Dr. Michael E. Carey's research project, "An Experimental Brain Missile Wound; Ascertaining Pathophysiology and Evaluating Treatments to Lower Mortality and Morbidity", and to report the results of the review to him.

Members of the committee were:

H. Douglas Braymer, Ph.D., Chairman, Professor of Microbiology and Acting Vice President of Academic Affairs, LSU System
Jack P. Strong, M.D., Professor and Head, Department of Pathology, LSU School of Medicine
Austin J. Sumner, M.D., Professor and Head, Department of Neurology, LSU School of Medicine
Nicholas G. Bazan, M.D., Ph.D., Professor of Ophthalmology, Biochemistry, and Neurology, and Director, Neuroscience Center of Excellence, LSU School of Medicine
John J. Spitzer, M.D., Professor and Head, Department of Physiology, LSU School of Medicine
Mack A. Thomas, M.D., Chief of Anesthesiology, VA Medical Center
John R. Ruby, Ph.D., Professor of Anatomy and Associate Dean for Faculty Affairs, LSU School of Medicine

The committee discussed the appropriateness of using the cat as the experimental animal in this project. It was determined that the cat was the proper animal. This decision was based on the amount of white matter, the vascularization, and the size of the cat brain. In addition, the cat has been utilized over many years for physiological studies which supply a very substantial store of data base upon which Dr. Carey's investigation could be built.

The committee discussed the anesthesia used in the experimental protocol. It was indicated that all anesthetics interact with the central nervous system and that pentobarbital has as few deleterious effects as possible and, therefore, it is the most appropriate agent for the study.

EXHIBIT 1

2

Also, it was agreed that pentobarbital was the most humane drug because it induces a deep state of unconsciousness and, hence, the animal is unaware of the injury.

The committee found the experimental design to be suitable and the parameters being measured to be appropriate. There was a difference of opinion within the committee as to the productivity of the project. A majority of the committee found that the productivity was appropriate and expected for a project that started from a zero base and that was plagued with personnel and technical problems. Further, the majority expressed the opinion that a very impressive body of good data had been obtained by this program. The data appeared to be scientifically important and potentially very valuable for applicability to human pathology. Several more publications should result from these data. Two of the sever members of the committee expressed the opinion that there should have been greater productivity.

The committee concluded that the project addressed critical scientific problems and that Dr. Carey's approach was well conceived, proper, and made maximum effort to protect the welfare of the animals. Further, it was the committee's opinion that Dr. Carey's project had received unwarranted and distorted coverage in the news media. Some members of the committee expressed grave concern about the political activity associated with this project and how such activity may affect the biomedical research community in the future. The committee was adamant in the opinion that the project should be continued and that the University should take the position of a strong defense on behalf of Dr. Carey.

EXHIBIT 1

3

Signatures of Committee to Review Dr. M. E. Carey's Research:

H. Douglas Braymer, Chair

Nicolas G. Bazan

John R. Ruby

John J. Spitzer

Jack P. Strong

Austin J. Sumner

Mack A. Thomas

EXHIBIT 2

THE AMERICAN ASSOCIATION OF NEUROLOGICAL SURGEONS

(FOUNDED AS THE HARVEY CUSHING SOCIETY IN 1931)
22 SOUTH WASHINGTON STREET, SUITE 100, PARK RIDGE, ILLINOIS 60068
PHONE: (708) 692-9500 FAX (708) 692-2589

March 29, 1990

Michael E. Carey, MD
Professor of Neurosurgery
Louisiana State University Medical Center
1542 Tulane Avenue
New Orleans, LA 70112

Dear Dr. Carey:

The American Association of Neurological Surgeons has completed its review of the research project on missile wounds of the brain, conducted at Louisiana State University Medical Center. Accordingly, I am enclosing the Association's Statement, containing the conclusions and recommendation.

Yours very truly,

Albert L. Rhoton, Jr., MD
President

EXHIBIT 2

Statement of the
AMERICAN ASSOCIATION OF NEUROLOGICAL SURGEONS
Regarding Certain Research Conducted at
Louisiana State University Medical Center

March 30, 1990

Michael E. Carey, MD, of Louisiana State University Medical Center, received funding by the Department of Defense to conduct research on missile wounds of the brain. Government interest in this type of research stems from the fact that despite the remarkable advances in medicine, the mortality rate from battle-related brain injury has remained essentially the same since World War II. Equally significant is the fact that the civilian population in the United States suffers thousands of deaths annually from gunshot wounds to the head.

This research was approved by local and federal scientific peer review. Subsequently, when his research funding was suspended, Dr. Carey requested that the American Association of Neurological Surgeons review this research project. Consistent with its commitment to the improvement of patient care through neuroscience research, the Association agreed to do so. In the course of this review, Dr. Carey's research summaries, publications, and reports were studied. He, his staff, and key personnel of the LSU School of Medicine were interviewed. Dr. Carey's laboratories and the LSU animal care facilities were inspected. As a result, several conclusions were reached:

1. The investigative team has the background, physical facilities, and equipment to conduct this research. This team is the only one currently studying brain missile injury in the United States.

2. The work which this laboratory team has done, and is planning to do, does not duplicate the comparatively little research previously conducted and reported in this area.

3. All studies have been performed with adequately anesthetized cats and rats. The anesthetic technique used is humane and does not invalidate the experimental modelling.

4. The experimental model chosen is appropriate to study missile injury of the brain. Since such wounds produce primary and secondary local and systemic events, computer modelling cannot reproduce the widespread response to injury necessary to understand and treat this type of wound.

5. Dr. Carey's studies to date have been well-planned and well-executed. While the team has concentrated on studying the pathophysiological effects of experimental brain missile injury, the model is developed to the point where treatment trials can be initiated to search for ways of improving the outcome from missile injuries to the brain.

Based upon these conclusions, it is recommended that this research be permitted to continue.

EXHIBIT 3

Mayo Clinic   Rochester, Minnesota 55905   Telephone 507 284-2511

John D. Michenfelder, M.D.
Department of Anesthesiology

November 3, 1989

Michael Carey, M.D.
LSU Medical Center
Department of Neurosurgery
1542 Tulane Avenue
New Orleans, LA 70112

Dear Doctor Carey:

In response to your request, let me briefly summarize the effects of pentobarbital anesthesia on cerebral physiology and compare and contrast that with other anesthetic techniques. Depending on depth of anesthesia, barbiturates will consistently decrease both cerebral blood flow and cerebral metabolic rate but do not uncouple the relationship between the two. Neither do barbiturates alter autoregulation nor do they abolish CO_2 reactivity (although the slope of the reactivity may be flattened somewhat). Barbiturates differ in their capacity to act as free radical scavengers such that thiopental does offer that potential whereas pentobarbital (the drug you are using) has no free radical scavenging potential (see Smith DS, Rehncrona S, Siesjo BK: Anesthesiology 53:186, 1980). There is some evidence that barbiturates offer brain protection in circumstances of regional ischemia and this is generally thought to be on the basis of reduced metabolic demand. Depending on the questions being asked, pentobarbital may be the ideal anesthetic for certain animal investigations. The commonly used volatile anesthetics all cause increased blood flow with decreased metabolism thus uncoupling the relationship between blood flow and metabolism and all interfere with autoregulation. As such, they are prone to increase intracranial pressure whereas barbiturates are prone to decrease intracranial pressure. Unfortunately, I know of no anesthetic technique that can predictably not alter either blood flow, metabolism, or normal cerebral physiology. It is a dilemma that all animal investigators face when they are studying questions relating to brain pharmacology, physiology, and pathophysiology. Thus, it is imperative to understand the effects of the background anesthetic chosen and to decide whether or not those effects are important in relation to the question being asked. Selection of a background anesthetic for animal research of this sort is always some kind of a compromise and thus, depending on ones motivation, fault can always be found with whatever choice is made. As per your request, I enclose a copy of my CV and bibliography.

Sincerely,

John D. Michenfelder, M.D.
Professor of Anesthesiology
Mayo Medical School
Chairman,
Division of Anesthesia Research

The University of Iowa

EXHIBIT 4

Iowa City, Iowa 52242

College of Medicine
Department of Internal Medicine

319/356-2883

1847

November 10, 1989

Michael E. Carey, M.D.
Professor of Neurosurgery
School of Medicine in New Orleans
Louisiana State University
Medical Center
1542 Tulane Avenue
New Orleans, LA 70112-2822

Dear Dr. Carey,

I am responding to your request for my opinion about the use of pentobarbital and inhalation anesthetics in studies of autoregulation of cerebral blood flow in cats.

We have used pentobarbital to study autoregulation of cerebral blood flow in rats, and autoregulation definitely is preserved. Some of the classic studies of mechanisms of autoregulation in the cerebral circulation have been performed in cats that were anesthetized with pentobarbital. I think that pentobarbital is certainly appropriate for studies of autoregulation in cats. Barbiturates and many other anesthetics tend to reduce cerebral blood flow, because they reduce cerebral metabolism, but autoregulation clearly is preserved in cats that are anesthetized with pentobarbital. Frankly, I am surprised that a question has been raised about the use of pentobarbital, because it is a very reliable, effective anesthetic.

We have used halothane in experiments in dogs, and it produces pronounced increase in cerebral blood flow. Halothane would be an acceptable anesthetic, but I would prefer pentobarbital in studies of autoregulation.

Sincerely,

Donald Heistad

Donald D. Heistad, M.D.
Professor of Medicine and Pharmacology

DDH:mmk

525 EAST 68th STREET, NEW YORK, N.Y. 10021

THE NEW YORK HOSPITAL-CORNELL MEDICAL CENTER

EXHIBIT 5

FRED PLUM, M.D., CHAIRMAN
ANNE PARRISH TITZELL PROFESSOR OF NEUROLOGY
DEPARTMENT OF NEUROLOGY AND NEUROSCIENCE
CORNELL UNIVERSITY MEDICAL COLLEGE
NEUROLOGIST-IN-CHIEF
THE NEW YORK HOSPITAL
(212) 746-6141
FAX (212) 746-8532

November 9, 1989

Dr. Michael Carey
School of Medicine in New Orleans
Louisiana State University
Medical Center
1542 Tulane Avenue
New Orleans, LA 70112-2822
Fax#: 504-568-4843

Dear Dr. Carey:

As you requested, I have reviewed the possible effect of the use of pentobarbital sodium on the outcome and interpretation of your study published in J. Neurosurgery, 1989, 71:754-764. In my opinion, the type and amount of anesthetic in no way invalidates the outcome or interpretation of the experiments.

Sincerely,

Fred Plum, M.D.

FP/moc

EXHIBIT 6

**LOUISIANA STATE UNIVERSITY
MEDICAL CENTER**
1542 Tulane Avenue
New Orleans, LA 70112-2822
Telephone: (504) 568-6090

Division of Animal Care

July 27, 1990

To: Michael Carey, M.D.
Professor, Department of Neurosurgery

From: Salvador G. Longoria, D.V.M.
Staff Veterinarian

I wish to clarify that I never told any member of the GAO or any other person that the animals from the brain wound project experiment experienced pain.

I did not use analgesia on these cats because they were not in pain and I have already stated that no analgesia was used.

S.G. Longoria D.V.M.

School of Allied Health Professions   School of Graduate Studies   School of Medicine in Shreveport

EXHIBIT 7

**LOUISIANA STATE UNIVERSITY
MEDICAL CENTER**
1542 Tulane Avenue
New Orleans, LA 70112-2822
Telephone: (504) 568-6090

Division of Animal Care

November 6, 1989

To: Michael Carey, M.D.
Professor, Dept. of Neurosurgery

From: Salvador Longoria, D.V.M.
Division of Animal Care

For Your Information:

On March 22, 1989 I had to drain an infected Pinna from the ear of your cat No. 1684.

On March 23, 1989 the cat seemed to act as though it were in pain so I gave him a dose of Butorphanol Tartrate (Torbutrol). It was not repeated because I did not consider it necessary. This was the only cat to which Butorphanol Tartrate was given.

School of Allied Health Professions School of Graduate Studies School of Medicine in Shreveport
School of Dentistry School of Medicine in New Orleans School of Nursing

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Major Contributors to This Report

Human Resources Division, Washington, D.C.

Susan D. Kladiva, Assistant Director, (202) 426-1357
Murray Grant, M.D., Chief Medical Advisor
Robert F. Gerkin, Evaluator-in-Charge
Linda O. Demoret, Evaluator

Glossary

Anesthesia Box	A covered aquarium-like tank in which the animal is placed while an inhalational anesthetic agent is introduced. The animal is removed from the box after it is under a correct plane of anesthesia.
Apnea	Respiratory arrest.
Arterial Blood Pressure	The pressure exerted by the circulating blood on the walls of the arterial blood vessels, produced by the pumping action of the heart.
Audio-Evoked Potential	Following a sound stimulus, electrical impulses are recorded from the audio centers of the brain or general increases in electrical activity produced by sound stimulus.
Autoregulation	The ability of the brain to control the blood flow into and out of its regions.
Balanced Anesthesia	The use of a combination of drugs to produce general anesthesia. The combinations are additive; therefore, the dose of a single drug or one of the drugs used reduces the side effects of the second drug.
Barbiturate	Any derivative of barbituric acid. Barbiturates are used as hypnotic and sedative drugs. Modifications in their structure influence the potency and rapidity of their effects. The depressant effects of these drugs are exerted on the higher centers of the brain.
Blood-Brain Barrier (BBB)	As defined by the research team, the functional barrier between the brain capillaries and the brain tissue that allows some substances from the blood to enter the brain rapidly and other substances slowly or not at all.
Blood Gases	Oxygen and carbon dioxide concentrations that are normally found in the blood.

Bradycardia	Slower-than-normal heart rate.
Butorphanol Tartrate	An analgesic drug given to relieve pain.
Cannula	An artificial tube of various sizes and shapes for insertion into a body cavity, an artery, or the trachea.
Catecholamine	Any one of a group of natural substances released by the body as a result of stress or injury, including epinephrine, norepinephrine, and dopamine.
Centigrade (C)	A temperature scale in which 0° represents the ice point and 100° the boiling point.
Centimeter (cm)	A unit of distance equaling one hundredth of a meter, equivalent to .3937 inch.
Cerebral Blood Flow (CBF)	The rate, in milliliters per minute, at which blood flows through the brain, measured by the rate of diffusion of inert gases (nitrous oxide, krypton) into the brain. Approximate value of CBF in normal people is 750 ml per minute.
Cerebrospinal Fluid (CSF)	The fluid within the cerebral ventricles and between the arachnoid membrane and pia mater of the brain and spinal cord.
CMR	Cerebral metabolic rate.
CRISP	Computer Retrieval of Information on Scientific Projects from the National Institutes of Health, Bethesda, Maryland.
Dexamethasone	A potent anti-inflammatory adreno/glucocortical steroid.

Dimethyl Sulfoxide (DMSO)	A colorless liquid, miscible with water. Rapidly absorbed through intact skin, it has local analgesic and anti-inflammatory activity.
DTIC	Defense Technical Information Center, located at Cameron Station, Virginia, indexes DOD research projects and reports on research projects.
Dura Mata	Layer of tissue that encloses the brain (brain cover).
Edema	Effusion of fluid into the interstices of cells in tissue spaces or into body cavities; swelling with fluid.
Electrocardiogram (ECG or EKG)	A graphic record, made by an electrocardiograph, of the electrical forces that produce the contraction of the heart. A typical normal record shows P, Q, R, S, T, and U waves.
Electroencephalogram (EEG)	A graphic record of the minute changes in electric potential associated with the activity of the cerebral cortex, as detected by electrodes applied in the surface of the scalp.
Electrolytes	A conducting medium in which the flow of current is accompanied by the movement of ions.
Energy	The capacity to do work; the property of a system that diminishes, when the system does work on any other system, by an amount equal to the work so done. Calculated by this formula: $E=1/2mv^2$.
Energy of Deposit	The difference between the missile's energy on entering and exiting the brain.
Euthanize	The intentional bringing about of an easy and painless death.

Evan's Blue Dye	A substance used to study gross (qualitative) changes in capillary integrity. Dye will diffuse into tissue upon rupture of a capillary.
Fix-Perfusion	To preserve and fix an organ of the body by the infusion (perfusion) of a fixative, such as formaldehyde.
Free Radical	A nonionic compound, highly reactive and of relatively short life, in which the central element is linked to an abnormal number of atoms or groups of atoms, and characterized by the presence of at least one unpaired electron.
General Anesthesia	A person or an animal in a state of altered body function resulting in insensibility to pain and a loss of consciousness, accomplished by the (1) injection of a combination of drugs or a single drug or (2) inhalation of an agent combined with oxygen.
Glycogen	A polysaccharide found in liver cells, all embryo tissues, testes, muscles, leukocytes, cartilage, or other tissues. It is formed from carbohydrates and is stored in the liver, where it is converted, as the system requires, into sugar (glucose).
Graded Response Model	A model producing different responses to injuries of increasing severity.
Halothane	A general anesthetic administered by inhalation.
Hematocrit	The percentage of whole blood cells in relation to the plasma component.
Hemodynamic	The study of the interrelationship of blood pressure to blood flow in the vascular system.
Heparin	An acid mucopolysaccharide acting as an antithrombin and antithromboplastin factor to prolong the clotting time of whole blood; it

occurs in a variety of tissues, most abundantly in the liver. Employed parenterally as an anticoagulant, in the form of the sodium salt.

HPLC	High performance liquid chromatography.
Hydrostatic	Part of the branch of hydraulics that deals with the properties and characteristics of liquids.
Hypercapnia	An excessive amount of carbon dioxide in the blood.
Hyperoxia	An excessive amount of oxygen in the blood.
Hypertension	Excessive tension or pressure, especially that exerted by bodily fluids such as blood, specifically, high blood pressure.
Hyperventilation	Abnormally rapid, deep breathing; overbreathing, usually due to anxiety, producing hypocapnia and symptoms of dizziness, paresthesia, and carpopedal spasm caused by the respiratory alkalosis that develops.
Hypocapnia	Subnormal concentration of carbon dioxide in the blood.
Hypotensive	Low blood pressure resulting from major loss of blood through hemorrhage.
Hypovolemic Shock	Shock caused by a reduced circulating blood volume which may be due to loss of blood or plasma as in burns, the crush syndrome, perforating wounds, or other trauma.
Hypoxia	Oxygen want or deficiency; any state wherein a physiologically inadequate amount of oxygen is available to, or utilized by, tissue without respect to cause or degree.

ILAR	Institute of Laboratory Animal Resources.
Inhalatant Anesthetics	Agents delivered to the animals' lungs in a carrier gas, such as oxygen or an oxygen-nitrous oxide mixture.
Intensive Care Unit (ICU)	An area within a hospital facility for patients whose health conditions require close medical attention, constant nursing care, and the use of complex medical equipment.
Intracranial Pressure (ICP)	Pressure within the cranium.
Intramuscular (IM)	Into a muscle mass, as to inject a drug into a muscle mass.
Intraperitoneal (IP)	Into the abdominal cavity.
Intravenous (IV)	Into a vein.
Ischemia	Local reduction in the blood supply to tissue due to obstruction of arterial blood inflow or vasoconstriction.
Isoflurane	An inhalant general anesthesia.
Joule	The meter-kilogram-second unit of work or energy; a missile weighing 31.7 milligrams moving at 178 meters per second has 0.50 Joules; the same missile moving at 210 meters per second has 0.70 Joules; at 238 meters per second, 0.90 Joules; at 297 meters per second, 1.40 Joules; and at 389 meters per second, 2.40 Joules.
Ketoacidotic	Acidosis produced by an increase in the blood of such ketone bodies as B-hydroxybutyric and acetoacetic acids.

Kilogram (kg)	A unit of mass and weight, equal to 1,000 grams (g).
Lactated Ringer's	A sterile solution of 0.6 g sodium chloride, 0.03 g potassium chloride, 0.02 g calcium chloride, and 0.31 g sodium lactate in sufficient water for injection to make 100 ml. Used intravenously as a systemic alkalizer and as a fluid and electrolyte replenisher.
Mannitol	A hexahydric alcohol from manna and other plant sources. It is used as a hypertonic solution, IV-administered to promote diuresis. Sometimes used to measure the rate of glomerular filtration and as an irrigating fluid in transurethral resection of the prostate; in pharmacy, used as a diluent.
Mean Arterial Blood Pressure (MABP)	Difference between the systolic and diastolic pressures.
MEDLINE	Medical Literature Analysis and Retrieval On-Line from the National Library of Medicine, Rockville, Maryland.
Methohexital Sodium	An ultrashort-acting barbiturate sold under the trade name of Brevital.
Milligram (mg)	A unit of weight equal to one thousandth of a gram.
Milliliter (ml)	A unit of capacity equal to one thousandth of a liter.
Millimeters of Mercury (mm/Hg)	The weight of a column of mercury 1 millimeter high, used to show the pressure of gases, blood pressure, and atmospheric pressure.
Missile Energy	E in Joules is calculated by $E=1/2\ mv^2$, where "m" represents the mass (in kilograms) of the sphere and "v" represents velocity (in meters per second) of the sphere.

Nitrous Oxide (N_2O)	A colorless gas used to produce anesthesia.
Normotensive	Normal blood pressure.
$PaCO_2$	The partial pressure of carbon dioxide in arterial blood.
PaO_2	The partial pressure of oxygen in arterial blood.
Pathophysiology	The study of the alterations in the physiological functions produced by a disease or pathological process.
pCO_2	Partial pressure of carbon dioxide, a method of expressing the level or amount of carbon dioxide.
PE 90	Size of a tube, usually made of polyethylene, used as a cannula for insertion in the cat's femoral artery.
Peer Review Process	The process by which research proposals are competitively evaluated through a discussion conducted by a review committee composed of scientists knowledgeable in the topic area. The committee evaluates each proposal to determine its scientific acceptability in areas such as research objective, scientific feasibility, investigator competence, and animal use.
Periosteum	A fibrous membrane investing the surfaces of bones, except at their points of tendinous and ligamentous attachment and on the articular surfaces, where cartilage is substituted.
Physiograph	Method of recording physiological values, for example, blood pressure and EKG.

Plasma Catecholamines	Any one of a group of natural substances found in blood released by the body as a result of stress or injury, including epinephrine, norepinephrine, and dopamine.
pO_2	Partial pressure of oxygen; a method of expressing the level or amount of oxygen.
Postoperative Care	Care given after a surgical procedure that includes monitoring—with attention to pain or discomfort and its alleviation—such factors as body temperature, fluid balance, and reflexes .
Prostaglandins	A group of powerful hormone-like chemicals found in all human and animal tissue other than red blood cells. One of several physiologically potent compounds, these chemicals have a unique structure containing 20 carbon atoms and are formed from essential fatty acids. The activities of these chemicals affect the nervous system, circulation, female reproductive organs, and metabolism.
Radioisotopes	A radioactive isotope, commonly of an element that is stable. By virtue of its radioactivity, a radioisotope is used either as a tracer added to the stable form of a compound (to follow the course of the compound in a particular sequence of reactions in living organisms or even in an inanimate system—as in this project to determine blood flow into and out of regions of the brain) or for the effect of its radiations (often diagnostic or therapeutic). Although certain isotopes of normally stable elements exist naturally in radioactive form, many are prepared only artificially, as by bombarding an element with neutrons, protons, deuterons, or alpha particles in a nuclear reactor or in an accelerating device such as the cyclotron or cosmotron; the bombarded element may form a radioactive isotope of the same element or of another element.
Sodium Bicarbonate	Used as a gastric antacid to combat systemic acidosis and to alkalinize urine.
Subcutaneous	Under the skin.

Tidal Volume	The volume of breath.
Trauma Model	Consists of the animal used, the method of preparing the animal for injury, and the method of causing a physical or mechanical injury for the purpose of studying the effects of the trauma on the animal or assessing the efficacy of various treatments. A valid model can be replicated over time with a high degree of consistency in results.
Velocity	The time rate of change of position of a body in a specified direction; rapidity of motion or operation; speed.

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